THESIS

DEVELOPMENT OF PORTABLE RECYCLED VERTICAL FLOW CONSTRUCTED WETLANDS FOR THE SUSTAINABLE TREATMENT OF DOMESTIC GREYWATER AND DAIRY WASTEWATER

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring, 2011

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ABSTRACT

DEVELOPMENT OF PORTABLE RECYCLED VERTICAL FLOW CONSTRUCTED WETLANDS FOR THE SUSTAINABLE TREATMENT OF DOMESTIC GREYWATER AND DAIRY WASTEWATER

Environmentally and economically sustainable wastewater treatment systems are more attractive as many countries and communities are becoming water scarce, heavily populated and decentralized. The reuse of low strength wastewaters, such as domestic greywater, is a critical tool in abating the effects of water scarcity and population growth. However, since current wastewater treatment systems are too resource and energy intensive to implement and maintain, many rural, decentralized, and impoverished communities cannot afford to treat wastewater for reuse or to meet environmentally sustainable discharge levels. As a result, bacterial, chemical and nutrient contamination of food crops irrigated with untreated wastewaters threaten community health, or disallow water recycling and reuse altogether. Surface and ground waters are contaminated by the discharge of untreated domestic and agricultural wastewaters as well. Degraded surface and ground waters can incur tremendous costs in effort to remediate the impact of eutrophication. In addition, global climate change is driving many large scale natural disasters which destroy wastewater treatment infrastructure, and drive chemical and fecal contamination of surface waters. A portable, low-cost,

temporary water treatment system delivered to a natural disaster area would limit the outbreak of water borne diseases, such as cholera, and provide a primary water treatment process to supply emergency drinking water.

Therefore, the development of a low-cost, low-technology, effective and sustainable wastewater treatment technology becomes a priority. Constructed wetlands have been used for many decades, throughout Europe, Great Britain, and the U.S., as low-tech primary and secondary treatment systems for industrial, agricultural and domestic wastewater. However, the widespread implementation of constructed wetlands in rural and impoverished communities is inhibited by the capital cost of constructed wetlands, which can become excessive due to the harvesting and transportation of sands and gravels used in the construction of the wetland bed. The use of recyclable materials in the construction of artificial wetlands has not been thoroughly studied. However, some evidence has been supplied to indicate that gravels and sands could be replaced with recyclable plastic without compromising treatment efficacy.

The purpose of this study was to develop and evaluate a portable, recycled vertical flow constructed wetland (RVFCW) built with recyclable polyethylene terephthalate (PET) plastic, and to compare this wetland's treatment efficiencies to constructed wetlands built with traditional gravels. The ability of the RVFCWs to effect contaminant levels would be evaluated during the treatment of domestic greywater (GW) and dairy wastewater (DWW), to fully understand the capacities and limitations of the RVFCW across an entire wastewater spectrum.

A total of four 1 m^2, portable, recycled vertical flow constructed wetlands (RVFCW) were built for this study. Two RVFCWs were built with recycled PET plastic as the primary wetland bed media, and two units were constructed with traditional volcanic tuff. Two sedimentation basins were built to provide wastewater flow to one of each type of RVFCW unit. The RVFCW units were dosed with 350 l d^{-1}, six times during a two month period for both greywater and dairy wastewater. Each RVFCW was equipped with a reservoir which received the effluent from the wetland. The wetland effluent was recirculated back to the wetland with a submersible pump for a period of 16 hr. Water samples were taken at four different locations within the treatment stream: at the wastewater source, sedimentation basin outflow, RVFCW unit outflow, and recirculation outflow. Each water sample was analyzed for the following parameters: total phosphate (TP), ammonia-nitrogen, nitrate-nitrogen, sulfate (for the GW stage only), total suspended solids (TSS), total dissolved solids (TDS), total organic carbon (TOC), total nitrogen (TN) (for DWW stage only), biochemical oxygen demand (BOD) (for DWW stage only), pH, total plate count (TPC), fecal coliforms (FC), and *Escherichia coli* (for DWW stage only).

The RVFCW units showed a 185±178% TP increase ($p = 0.0014$) during GW treatment, and a 17.2±30% TP decrease ($p < 0.0001$) during DWW treatment. The units achieved a 78.6±26% ($p < 0.0001$) and 70±11.7% ($p < 0.0001$) decrease in ammonia-nitrogen during GW and DWW treatment, respectively. Nitrate-nitrogen increased during GW and DWW treatment by 5887±2992% ($p < 0.0001$) and 2028±1476% ($p < 0.0001$), respectively. Total suspended solids concentration increased by 26±139% ($p = 0.0036$) during GW treatment, but decreased by 81±7.4% ($p < 0.0001$) during DWW treatment. Total dissolved solids concentration increased during GW and DWW treatment by 320±185 % ($p <0.0001$) and 26±41.2% ($p = 0.0319$), respectively. The units achieved a 52±19% decrease ($p < 0.0001$) in TOC concentrations during GW treatment, and a 68.1±8.8% decrease ($p < 0.0001$) during DWW treatment. Total nitrogen concentrations were analyzed for the DWW stage only, and decreased by 45.8±7.8% ($p < 0.0001$) during treatment. Biochemical oxygen demand was also analyzed during the DWW stage. The units achieved a 51±19.1% reduction ($p < 0.0001$) in BOD during treatment. pH values increased by 21±1.6% ($p < 0.0001$) during GW treatment, and increased by 9±2.1% ($p < 0.0001$) during DWW treatment. The units achieved a 2 log reduction ($p < 0.0001$) in TPC and a 3 log reduction ($p < 0.0001$) in FC, during GW treatment. During DWW treatment, the RVFCW units did not demonstrate a statistically significant increase or decrease in TPC, but did achieve a 0.4 log reduction ($p < 0.0001$) in FC. The RVFCW units achieved a 1 log reduction ($p < 0.0001$) in *E. coli*, during DWW treatment. Sulfate values were considered invalid, due to contamination of the RVFCW units by high sulfate water used to water the units during the pre-trial, growth period.

There was no statistical difference between the RVFCW unit types for all parameters except ammonia, nitrate and pH. The RVFCW units constructed with PET showed a 72.8±33% ($p = 0.0266$) and 65±11.7% ($p = 0.0411$) decrease in ammonia-nitrogen concentrations during GW and DWW treatment, respectively. Whereas, the RVFCW units constructed with volcanic tuff achieved an 84.5±20% ($p = 0.0266$) and 75±9.8% ($p = 0.0411$) reduction in ammonia-nitrogen concentrations during GW and DWW treatment, respectively. Also, the RVFCW PET units demonstrated a 4742±2526% ($p = 0.0155$) and 1449±1069% ($p < 0.0001$) increase in nitrate-nitrogen concentrations during GW and DWW treatment, respectively. The RVFCW VT units demonstrated significantly a higher increase in nitrate-nitrogen concentrations, with a 7032±3207% ($p = 0.0155$) increase during GW treatment, and a 2607±1605% ($p < 0.0001$) increase during DWW treatment. The final pH values for the RVFCW PET units were 8.23 ($p = 0.0132$) during GW treatment, and 8.2 ($p = 0.0099$) during DWW treatment. Whereas the final pH value for the RVFCW VT units was 8.32 during GW treatment ($p = 0.0132$) and 8.38 during DWW treatment ($p = 0.0099$).

The results of this study indicate that, under current design parameters, the RVFCW units are suitable for remediation of bacterial contamination in greywater, and that plastic PET can be successfully used as a construction medium without compromising treatment efficacy. The results also indicate that, under current design parameters, the RVFCWs are not entirely suitable as a holistic treatment system for greywater if nutrient and solids reduction is a priority. However, the RVFCW units would perform well as a primary or secondary treatment system for high strength agricultural wastewaters, if nutrients and solids reduction is a priority. The RVFCW design would be an unsuitable stand alone treatment system for wastewaters characterized by elevated concentrations of nitrate-nitrogen. However, the RVFCW PET unit outflow nitrate-nitrogen concentrations suggest that, with some design modifications, these units could be effectively used as stand-alone treatment systems for agricultural wastewaters.

Overall the systems performed well, indicating that the RVFCW units would be a viable, low-cost, and effective wastewater treatment system for the recycling and reuse of greywater, and the remediation of agricultural wastewaters prior to surface water discharge. Polyethylene terephthalate plastic can replace gravel as a primary bed media without impairing treatment efficiencies, thereby drastically reducing the capital cost of artificial wetland implementation.

ACKNOWLEDGEMENTS

Thanks to Dr. Lawrence Goodridge for giving me the opportunity to go beyond the bounds of traditional Food Safety. Also, thanks to Dr. Terry Engle, Dr. Sybil Sharvelle, and Dr. S. Ranil Wickramasinghe for their constructive criticism and guidance. Thanks to Bledar Bisha for his professional advice and help and a very special thanks to my assistants in the laboratory, Kris Bruun and Katie Fialko.

DEDICATION

This thesis is dedicated to my mother, Susan Melcher, her chap Tom Swan, and my very patient husband, Kris Bruun. Also, to my grandmother, Alice Melcher, one of the finest teachers I have ever known.

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OBJECTIVE OF THESIS

I. To develop and evaluate a wastewater treatment system to treat greywater and agricultural wastewater for reuse with a recycled vertical flow constructed wetland as the design model.

II. To evaluate the viability of utilizing recycled polyethylene terephthalate plastic as a wetland bed media by comparing treatment performances between wetlands constructed with plastic media and wetlands constructed with volcanic tuff.

Chapter 1

Introduction

1. Introduction

As global water resources become depleted and polluted by an increasing human population, there is an ever growing need for sustainable, effective and economically viable wastewater treatments systems (Wilderer, 2005). To help alleviate the problem of potable water scarcity, these treatment systems must also incorporate mechanisms for wastewater recycling and reuse (Denny, 1997). Greywater in particular, which comprises about 50-75% of domestic water consumption, can be treated to various levels of quality and reused for irrigation and bathing (Eriksson et al., 2002). However, in many places, greywater is being discharged without treatment directly into surface waters or used as irrigation water (Dallas and Ho, 2005). These practices not only exacerbate water shortages when water is discharged unused, but lead to a significant deterioration of local soil and water quality when the water is reused without prior treatment (Gross et al., 2007, Maimon et al., 2010, Travis et al., 2010). In addition, agricultural runoff and wastewaters have contributed substantially to surface water degradation and eutrophication around the world. The excess nutrients found in agricultural runoff result in loss of biodiversity in waterways, habitat degradation, shifts in food chain structure, fisheries impairment, and outbreak of nuisance aquatic species (Moore et al., 2010).

Agricultural runoff is considered non-point source pollution and is largely unregulated in the United States, and therefore enters the environment without treatment (Novotny, 2003). Constructed wetlands have been used for decades throughout the United States and Europe for the treatment of both agricultural wastewater and greywater (Brix et al., 2007, Rousseau et al., 2007, Kadlec and Scott, 2009). However, this technology is still vastly underused in industrialized countries and is almost non-existent in developing nations where water treatment and recycling could be most beneficial.

1.1. Problem Statement

1.1.1. Global Sanitation and Greywater

On September 8, 2000, the United Nations (UN) met in general assembly to reestablish their commitment of service to the most vulnerable of the world's people, especially children (United Nations, 2008). Eight goals were drawn up to affirm this commitment: eradicate extreme poverty and hunger, achieve universal primary education, promote gender equality, reduce child mortality, improve maternal health, combat HIV/AIDS, malaria and other diseases, ensure environmental sustainability, and develop a global partnership for development (United Nations, 2008). These goals are titled as Millennium Development Goals (MDG), and are tremendously ambitious, with a definitive pyramidal hierarchy (United Nations, 2008). At the base of this pyramid is MDG target 7c: to reduce by half, the proportion of people without sustainable access to safe drinking water. The effects of poor water quality and drinking water scarcity, is at the root of poverty, child mortality, and disease (United Nations, 2008). Unfortunately, according to the 2008 MDG report, the UN is a long way from achieving the 7c target with 2.5 billion people still without adequate access to sanitation and clean water (United

Nations, 2008). Currently, in developing nations, 75% of industrial wastewater and 90-95% of raw sewage wastewater is discharged into surface water bodies without any treatment (United Nations, 2007). In most developing nations people withdraw drinking water from the same water body they use for excreta disposal (United Nations, 2008). Also critical to recognize, is target 7c, which specifies the need to provide drinking water with sustainable methods (United Nations, 2008). Water scarcity is a function of economics, pollution, and disruption of natural water cycles (Hunt, 2004). A 2005 report, issued by the United Nations Environmental Programme, estimated that 40% of the world's population will live in water scarce regions by 2015 (United Nations, 2005). This amounts to as many as 1.8 billion people living in water scarce regions, which are already undergoing huge pollutant contaminations, and excess water withdrawal (United Nations, 2008). Clearly, target 7c will be much harder to achieve if water supply is insufficient to meet basic needs.

The main impediment the UN will encounter during the effort to halve the proportion of the world's people without access to safe drinking water by 2015 is cost. The World Bank estimates that from the time the UN met in 2000, roughly 150 billion US$ would need to be spent every year to bring sanitation systems similar to those used in industrialized nations to those nations currently without such infrastructure (United Nations, 2008). According to data attained from the World Health Organization's website, approximately 45.38% of the world's population did not have access to adequate sanitation in 2000 (WHO, 2010). By 2004, that number had dropped to 42.82% (United Nations, 2008). The monetary resources needed to equip developing nations with sanitation systems identical to those found in industrialized nations will not be distributed in time to meet the UN's goal to reduce the population without access to adequate sanitation by half, by the year 2015. Nor could sanitation systems utilized in industrialized nations meet the needs of the many people who currently reside in water scarce regions, as the primary wastewater treatment systems employed are flushing sewers (Hunt, 2004).

Flushing sewer systems are parts of standard disposal-based linear systems used in industrialized nations throughout the world (Hunt, 2004). These systems add greater water demand on already water taxed countries, as well as greatly increase the treatment cost due to the larger volume of water that would be used for flushing (Hunt, 2004). Also, these systems are large, expensive, water and nutrient wasteful, and require extensive maintenance as well as a highly skilled and trained workforce to function properly (Hunt, 2004). In addition, these treatment systems often disrupt the local, natural water cycle in a supply watershed basin by requiring extensive water transportation courses, and storage basins (Hunt, 2004). Flushing sewer systems also inhibit the conservation of water and nutrients. Sustainable practices are now recognized to be based on the restoration, or even mimicry, of natural water cycles where they have been disrupted (Hunt, 2004).

Wastewater treatment facilities in developing countries can only be considered sustainable if they are cost-effective, technologically appropriate, and minimally disrupt local, natural water cycles. Constructed wetlands can provide a treatment system capable of meeting all these criteria (Denny, 1997).

1.1.2. Agricultural Wastewater

Agricultural activities in the United States are a primary source of surface water pollution in the United States. Irrigated and non-irrigated cropland, concentrated animal feeding operations and animal production on range and pastureland are all sources of wastewaters, polluted runoff and irrigation return flows (Novotny, 2003). The pollutants most commonly found in agricultural wastewaters and agriculturally impacted waters are: excess phosphorus, nitrogen, sediment, pesticides, pharmaceuticals, and pathogens. (Novotny, 2003, Muller et al., 2000, Pruden et al., 2006, Koplin et al., 2002). The agricultural pollutants of particular concern are excess nutrients. Phosphorus transport in runoff from agricultural land and animal feeding operations are considered one of the major factors controlling eutrophication of U.S. freshwaters and the Gulf of Mexico (Sharpley, 1980, Moore et al. 2010, Mitsch et al. 2001). In 2008, Dodds et al. (2008) reported that the annual cost of eutrophication in the United States was $2.2 billion, and that the ability to control eutrophication relied on the ability to control and mitigate total phosphorus and total nitrogen nutrient loads.

For most agriculturally impacted water and agricultural wastewaters, conventional treatment options are either economically prohibitive or impractical for a decentralized location. Interest in treating agriculturally impacted waters with constructed wetlands gained popularity in the United States during the 1980s (EPA, 1988). This was due to the following benefits associated with constructed wetlands: the natural treatment mechanisms provided by aquatic plants as nutrient sinks and buffer zones; the aesthetic and environmental benefits to humans and wildlife associated with the preservation and reconstruction of wetlands; and the rapidly increasing costs of constructing and operating a traditional wastewater treatment facility (EPA, 1988). In addition, constructed wetlands often have a higher tolerance for fluctuations in contamination levels and loading rates than conventional treatment systems, a particular benefit as agricultural pollutant loads are often event driven (Novotny, 2003).

1.1.3. Emergency Water Treatment

In December 2004, a powerful tsunami hit the coasts of Thailand, Indonesia, Sri Lanka, and parts of the West African coast. More than 280,000 people were killed, 1.5 million people were displaced, and domestic infrastructure for millions was destroyed (Brix et al., 2007, Steckley and Doberstein, 2010). Utility networks, roads, electrical power supplies, wastewater drainage systems, and water supply systems were completely destroyed (Brix et al., 2007). Foreign aid to rebuild wastewater management services in parts of Thailand was not received for over a year. As of 2007, more than ninety municipal wastewater treatment plants in Thailand were still non-functioning (Brix et al., 2007).

Hurricane Katrina, a category 4 hurricane, destroyed large swaths of domestic infrastructure throughout Mississippi and Louisiana (Smith and Rowland, 2010). In New Orleans, several storm levees broke, flooding residential areas, killing over 1,000 people and leaving thousands of others stranded for days awaiting rescue (Gheytanchi et al., 2007). The flood waters contained high concentrations of lead, mercury, motor oil, arsenic, benzo[a]pyrene, DDD (dichlorodiphenyldichloroethane), and fecal material (Furey et al., 2007, Suedel et al., 2007). Maximum flood levels were reached September 2nd, 2005 three days after the levees broke. In total, the U.S. Army Corps of Engineers pumped 847,932,243 m^3 of contaminated flood water out of the residential areas of New Orleans into the surrounding marshes (Smith and Rowland, 2010).

Emergency water treatment systems capable of performing appreciable primary treatment for high volume flow are currently unavailable. When hydrologic disasters strike, stranded residents have to rely on emergency water trucks and bottled water to be driven or flown into the effected region. In addition, wastewater treatment infrastructure can take months to repair, leaving citizens without access to adequate sanitation for long periods, exposing the immune-compromised to biologically contaminated water. Portable constructed wetlands have not been investigated as potential primary and secondary treatment systems for areas with impaired wastewater facilities, despite certain subsurface flow constructed wetland designs which would facilitate portability. If portable constructed wetlands can be transported to areas with destroyed or impaired wastewater treatment, citizens of the effected area would not be left exposed to untreated wastewaters.

1.2. Objective

The objective of this research is to develop an environmentally and economically sustainable wastewater treatment option for developing nations, decentralized communities and temporary wastewater treatment in the event of infrastructural damage due to hydrologic disasters. The wastewater treatment option has to be environmentally sustainable, economically viable for impoverished communities, and require less resources and surface area. In addition, the wastewater treatment option has to be capable of treating a wide range of wastewaters from relatively low strength domestic greywater to high strength dairy wastewater, without compromising treatment efficacy for either wastewater. A recycled vertical flow constructed wetland design by Gross et al. (2007) was chosen as a model for this research. The system design presented by the authors, best fit the parameters required to fulfill this research objective. The design was manipulated to investigate the potential for using recycled plastic in place of traditional bed media, in an effort to create a constructed wetland requiring less capital to build.

Chapter 2

Literature Review

2.1 Definition of Constructed Wetlands

Constructed wetlands (CWs) can be defined as artificially created ecosystems with partially to completely saturated soils planted with submergent, emergent or floating macrophytes or a combination of the three (Kadlec and Scott, 2009). Constructed wetlands are generally recognized to be efficient water purification systems and nutrient sinks, and have been used in conjunction with, or in place of, traditional wastewater treatment mechanisms (Moshiri, 1993). Constructed wetlands can serve as primary, secondary or tertiary water treatment. Traditional mechanisms for these levels of water treatment start with: primary clarifiers for removal of coarse, minimally decomposable organic solids and inorganic solids; facultative ponds, aerated lagoons, activated sludge, trickling filters or rotating biological reactors for the removal of dissolved organic matter and other wastewater solids which passed through the primary treatment mechanism; and chemical precipitation, ultrafiltation, reverse osmosis or ion exchange for the removal of those water contaminants which have not been reduced to adequate discharge levels during primary and secondary treatment (Kadlec and Scott, 2009). Constructed wetlands have been shown to be capable of achieving excellent removal efficiencies for wastewater pollutants and water contaminants, and therefore a viable replacement for many of the traditional processes which, require extensive capital and annual expense to maintain, need trained personnel to operate, and generate reject waste streams which require additional chemical treatment (Kadlec and Scott, 2009). Presently, in the United States and other industrialized nations, CWs are employed largely as secondary treatment for small communities, and as additions to aged or overloaded municipal secondary treatment plants, as well as a solids trapping system for facultative lagoons. Constructed wetlands have also served as tertiary treatment systems for compliant discharges in the event of regulatory changes (Kadlec and Scott, 2009).

2.1.1. Types of Constructed Wetlands

Current wetland designs are divided into two primary categories: surface flow and subsurface flow. Surface flow, or free water surface (FWS) wetlands have open water areas and typically contain all three types of wetland macrophytes, including submergent, emergent and floating (Kadlec and Scott, 2009). In FWS wetland systems there is minimal interaction at the soil-water interface, with the primary removal mechanism achieved by settling, adsorption and plant uptake (Kadlec and Scott, 2009). The air-water interface of FWS CWs create abundant opportunity for the volatilization of compounds into the atmosphere, as well as the exchange of oxygen and carbon dioxide (Moshiri, 1993).

Subsurface flow constructed wetlands (SSFCWs) may be constructed as one of two hydrologic modes: vertical or horizontal. In both, water is kept below the surface with water flowing either horizontally or vertically through the wetland bed media (Kadlec and Scott, 2009). Typically these wetlands are constructed with sandy loams, sands, pea gravel, and coarse gravel. The primary difference between the two hydrologic schemes is the presence or absence of oxygen (Kadlec and Scott, 2009). Subsurface vertical flow (SSVF) constructed wetlands are characterized by an abundance of oxygen. Soil pores are intermittently filled and drained of water which creates an aerobic environment for the facilitation of certain biochemical reactions, such as nitrification, and solids degradation (Kadlec and Scott, 2009). Subsurface horizontal flow (SSHF) constructed wetlands are characterized by strongly anaerobic, or anoxic, conditions within the wetland bed medium. In these conditions, oxygen transfer is limited at the soil-air interface which facilitates anerobic biochemical processes such as the denitrification of nitrate to elemental nitrogen, and the reduction of sulfate to hydrogen sulfide (Kadlec and Scott, 2009).

Vertical flow constructed wetlands can be further divided into subtypes, or modes. They may be operated in the following modes: intermittent downflow, unsaturated downflow, saturated up- or downflow, and tidal flow (Kadlec and Scott, 2009). Intermittent downflow is characterized by flooding the surface of the wetland for brief intervals. Unsaturated downflow is characterized by distributing water evenly across the surface of the wetland bed media which then trickles through the media and is often operated in a recirculation flow mode (Kadlec and Scott, 2009). Saturated up- or downflow utilizes continuous downward or upward flow to maximize soil saturation. Finally, tidal flow, or fill and drain flow utilizes a cyclic fill and drain flow pattern. Water is distributed into the wetland from the bottom, fills the wetland bed matrix, then following a holding period, is released (Kadlec and Scott, 2009).

2.1.2. Constructed Wetland Fate Processes

All constructed wetlands are able to alter the quality of water and wastewater influent by six fate processes, including sedimentation, adsorption/precipitation, macrophyte and microorganism transformation, chemical reactions, natural decomposition, and volatilization (Moshiri, 1993).

Many critical water pollutants partition strongly to suspended and dissolved solids, such as metal cations, ammonia, bacteria, and a variety of hydrophobic compounds such as detergents and organic solvents (Novotny, 2003). If a high percent of suspended and dissolved solids can be removed from the water and wastewater, those contaminants partitioned with the solids will be necessarily removed as well (Kadlec and Scott, 2009, Novotny, 2003). In FWS constructed wetlands, hydraulic conditions allow a large percent removal of solids through sedimentation with low flow velocities (Kadlec and Scott, 2009). Natural water turbulence within the wetland facilitates even distribution of settled total solids through the entire wetland area (Moshiri, 1993). Subsurface flow constructed wetlands achieve solids removal through a mechanism more accurately identified as interception (Kadlec and Scott, 2009). Suspended solids will be trapped within the wetland bed matrix as the water flows through the soil pores. Once intercepted by the bed matrix these trapped solids can be further reduced by aerobic decomposition, bacterial degradation or a combination of the two (Kadlec and Scott, 2009). Another form of sedimentation is the process of accretion (Moshiri, 1993). Plant matter and material native to the wetland will combine with wastewater solids trapped on top of and within the wetland bed matrix to form either movable sediments, or nonsuspendable accretions.

These accretions are typically stable and can resist extensive decomposition (Moshiri, 1993).

Related closely to the mechanism by which sedimentation functions as a pollutant control is adsorption and precipitation (Moshiri, 1993). Depending on wetland soil conditions, toxic metals can be reduced or oxidized to chemical forms which will partition heavily to the chemical constituents found naturally in the wetland sediments (Novotny, 2003).

Anaerobic and aerobic bacterial conversion of nutrients, chemicals and metal compounds is the primary mechanism by which those constituents are remediated in a wetland environment (Kadlec and Scott, 2009). The lifecycle of bacterial populations provides an assimilative, transformative and recycling effect on nitrogen, phosphorus and carbon (Moshiri, 1993). Wetland macrophytes provide nutrient and contaminant removal by uptake and assimilation of those constituents into plant tissues. Both plant and bacterial activity plays a critical role in oxygen consumption and transfer within the wetland sediments which drive both aerobic processes, such as nitrification, and anaerobic processes, such as denitrification and sulfate reduction, critical to altering influent water quality (Kadlec and Scott, 2009).

Chemical reactions in wetland ecology are driven by four factors, including dissolved oxygen concentration, oxidation-reduction potential, hydrogen ion concentration (pH), and alkalinity (Kadlec and Scott, 2009). These four factors are directly related to the rate at which many compounds are oxidized and reduced. The same elemental compound can be transformed from a toxic water pollutant to an environmentally inert molecule during an oxidation-reduction reaction (Moshiri, 1993).

For example, sulfate oxidation will occur in the presence of an external carbon energy source at a pH greater than 8, but only when the redox potential or pε (defined as the negative log of the electron concentration) is below 0. Redox potential drops as dissolved oxygen has been exhausted as an electron acceptor (Stumm and Morgan, 1996). The oxidation of sulfate, a potent plant and animal pollutant, to hydrogen sulfide facilitates the formation of insoluble iron sulfide precipitates (Kadlec and Scott, 2009). The extent to which hydrogen sulfide will form an iron sulfide is influenced by the amount of iron carbonate present in the water, which is governed partially by the alkalinity of the influent wastewater and the natural alkalinity of the wetland (Stumm and Morgan, 1996). These types of interactions between pH, pε, alkalinity and dissolved oxygen concentration govern the occurrence and rate of all biochemical and chemical transformations in a constructed wetland system (Kadlec and Scott, 2009). In addition to these chemical interactions, solar radiation has been shown to generate numerous photochemical reactions which cause degradation of a variety of organic compounds. (Moshiri, 1993)

Volatilization is the process by which compounds will transfer from a solid and liquid phase to a gaseous phase (Kadlec and Scott, 2009). Compounds such as ammonia, BTEX (benzene, toluene, ethyl benzene and xylenes), hydrogen sulfide, nitrogen gas, nitrous oxide and methane are all readily volatilized into the atmosphere (Novotny, 2003, Kadlec and Scott, 2009). Once a compound has been chemically transformed into a species that is readily volatilized, the water temperature, water turbulence, air temperature, and partial pressures of the compound in the water and air will dictate the rate at which mass transfer of the compound will occur at the water-atmosphere interface (Moshiri, 1993).

2.2. Case Studies

Used alone or in series with another type of wetland, a CW can achieve high enough removal efficiencies, with optimal design modifications and system development, to make CWs a viable, inexpensive and sustainable wastewater treatment option for decentralized communities, concentrated animal feeding operations, dairies, and impoverished communities.

For example, Aquilar et al. (2008) analyzed the effectiveness of CWs in remediating tannery wastewater in León Gto., Mexico. The wastewater, produced from 650 tanneries, was not recycled for reuse, nor could the water be discharged directly into lagoons or surface waters, due to the concentration of pollutants. Tannery wastewater is dominated by organic matter, chromium, nitrogen and sulfur compounds (Aquilar et al., 2008). In addition, tannery wastewater can contain a biochemical oxygen demand (BOD) concentration of 1600 mg l^{-1} (Aquilar et al., 2009). A three cell CW was built to receive wastewater from a tannery for one year. The CW was planted with both *Scirpus americanus* and *Typha spp.* Samples were taken over the course of one year, with sediment and water samples taken every four months at three different locations within each of the CW cells. The overall removal efficiencies for BOD, chromium, total kjeldahl nitrogen (TKN), and sulfate were all between 88% and 99%. In this study, the authors showed that *Scirpus* and *Typha* played a crucial and successful role in the attenuation of chromium and other chemicals found to be toxic to critical wetland microorganisms. A variety of denitrifiers, sulfur oxiding bacteria, and sulfate reducing bacteria were found in rhizosphere sediment profiles, such as, *Pseudomonas*, *Alcaligene*, *Ochrobactrum*, and *Acinctobacter.*

A 2008 paper by Riefler et al. detailed the ability of a five cell CW system to remediate acid-mine drainage (AMD) in Coshocton, Ohio. Acid - mine drainage is characterized by a combination of low pH and high concentrations of metal cations, which are extremely toxic to natural aquatic ecosystems. In most cases, preventing the formation of AMD is difficult, therefore the development of an effective remediation solution is critical. Remediating AMD requires processes which raise the pH and alkalinity. These processes facilitate the removal of dissolved metal cations. Riefler and colleagues (2008) focused on a series of anaerobic vertical flow CWs linked in series with aerobic wetlands so that an anaerobic vertical flow (AVFW) constructed wetland provided the inflow to an aerobic wetland which fed another AVFW, which fed a second aerobic wetland providing inflow to a final AVFW leading ultimately to a large retention pond. The series is called a successive alkalinity producing system (SAPS). Riefler et al. (2008) conducted a 20 month study of the SAPS providing treatment for an AMD source and found that the system generated 26 kg $CaCO_3$ d^{-1} of alkalinity, and captured 5 kg d^{-1} and 1.7 kg d^{-1} of iron and aluminum, respectively. Although the capacity of the wetland system was reached during the monitoring period, the authors maintained that, overall, the SAPS achieved excellent removal efficiencies and neutralization of pH.

In Limerick, Ireland, a hybrid reed bed system was built to receive and treat waste from a fertilizer plant which was located close to a river estuary (Cooper et al., 2010). Environmental regulations required the fertilizer plant to treat the wastewater produced from their plant to comply with discharge limitations for nitrate-nitrogen, ammonia-nitrogen, and phosphate, which were 50 mg l^{-1}, 2 mg l^{-1}, and 0.7 mg l^{-1}, respectively. The fertilizer plant was producing wastewater effluent at 60 mg l^{-1} of nitrate-nitrogen, 120 mg l^{-1} of ammonia-nitrogen, and 60 mg l^{-1} of ortho-phosphate. The CW was composed of a horizontal flow constructed wetland (HFCW) providing flow to a vertical flow constructed wetland (VFCW). Effluent from the VFCW was recycled back to the HFCW at a 4:1 ratio. Both wetlands were planted with *Phragmites australis.* The goal of the recycled hybrid system was to maximize the opportunity for nitrification and denitrifcation processes by providing optimal aerobic and anoxic conditions in succession. Cooper and colleagues (2010) reported an 81.8% removal of ammonia-nitrogen, and 75% removal of nitrate-nitrogen. Final effluent concentrations were 14 mg l^{-1} for ammonia-nitrogen, and 18 mg l^{-1} of nitrate-nitrogen. Phosphate removal was aided by chemical flocculation pretreatment, achieving a final effluent level of 0.5 mg l^{-1} of total phosphate (Cooper et al., 2010).

Ye at al. (2003) used wetland microcosms to determine whether or not CWs would be capable of receiving and treating high strength coal gasification wastewater. The authors reported that there had been no previous studies on CWs treating water with metal contamination as high as can be found in the wastewaters of a coal gasification plant. In addition, the wastewater contained the more toxic anion of selenium, selenocyanurate ($SeCN^-$). The authors constructed wetland microcosms with 14 different plant species and conducted a 54-d trial to determine the effluent concentration and total mass of four metals: selenium, arsenic, boron, and cyanide. The concentrations were found to be of 1.22 mg l^{-1} of selenium, 0.46 mg l^{-1} of arsenic, 46.5 mg l^{-1} of boron, and 2.16 mg l^{-1} of cyanide. This study found that of the fourteen wetland macrophytes used, only seven species survived, including cattail, *Thalia*, rabbitfoot grass, arrowhead, softstem bulrush, dwarf yellow iris, and golden reed. Of these seven, cattail, *Thalia*, and rabbitfoot grass appeared unaffected by the toxicity level of the influent wastewater. At the end of the 54-d period, effluent was at 0.51 mg l^{-1} for selenium, 0.46 mg l^{-1} for arsenic, 32.1 mg l^{-1} for boron, and 1.52 mg l^{-1} for cyanide. The authors then tested the toxicity of the effluent by submitting the CW effluent to a biomonitoring facility to be tested on fathead minnows. The biomonitoring facility reported that when grown in the presence of 100% effluent, the fathead minnows did demonstrate a slight degree of chronic toxicity. A chronic toxicity effect was not found in the minnows when they were grown in the presence of lower CW effluent concentrations (Ye et al., 2003).

Wood et al. (2007) utilized vertical flow reed beds to treat dairy wastewater in Sussex, England. Constructed in series with an intensive aeration pre-treatment system and a trickling bed filter (TBR), two reed beds received wastewater from a 400 hd dairy farm. The reed beds were used in rotation with one in use and the other resting. The influent wastewater quality, after pre-treatment with intensive aeration and a TBR, was analyzed for BOD and total solids (TS). Biochemical oxygen demand was reported at 21.9 mg l^{-1}, and TS was at 2089 mg l^{-1}. Averaged over two years of sample analyses, the reed beds achieved effluent rates of 11.7 mg BOD l^{-1} and 1851 mg l^{-1} for TS. The authors concluded that the reed bed system was an effective treatment option for dairy wastewater when operated as a polishing step after intensive aeration.

Nielsen (2007) studied the reduction of pathogenic microorganisms in an 11-year old CW system receiving sludge from a municipal wastewater treatment plant. The reed beds received a hydraulic loading rate of sludge at 60 kg dry solids m^{-2} yr^{-1}. The sludge was applied once or twice daily for a period of 7-8 days, followed by 55-65 days of rest. The author sampled sludge in the CW system on eight different days over a five month period, and analyzed the samples for *Salmonella, Enterococci*, and *E. coli*. The author found a 2 log reduction in *Salmonella*, a 5 log reduction in *Enterococci*, and a 6-7 log reduction for *E. coli*. The author indicated that the reduction in pathogenic microorganisms implied that the treated sludge from the CWs was better suited for reuse on agricultural lands than mechanically dewatered sludge.

The constructed wetlands detailed in the previous paragraphs were diverse in size, the types of macrophytes used, and the depth and type of media. They efficiently treated tannery wastewater, acid-mine drainage, fertilizer plant wastewater, coal gasification wastewater, dairy effluent, and municipal wastewater sludge (Aquilar et al., 2008, Riefler et al., 2008, Cooper et al., 2010, Ye et al., 2003, Wood et al., 2007, Nielsen, 2007). All of the constructed wetlands detailed performed effectively despite treating wastewaters with elevated heavy metal loading rates, toxic levels of selenocynurate, high biochemical oxygen demand, elevated total suspended solids concentrations, and excessive nutrient loadings. These case studies provide strong arguments for the effectiveness of constructed wetlands under diverse pollutant loading rates, conditions which can frequently inhibit treatment efficiencies in traditional wastewater treatment facilities (Droste, 1996)

2.3. Vertical Flow Constructed Wetlands

Of the three types of constructed wetlands, free water surface flow constructed wetlands (FWS CWs) are the least practical for use as an urban greywater recycling and purification system. Free water surface CWs are impractical due to the risk of exposure to microbial contamination at the open water surface. As a result, they are considered impractical for primary or secondary treatment of wastewater (EPA, 2008). Free water surface CWs would be particularly impractical for many developing nations as they would provide a substantial exposed water surface area for mosquito breeding (Dallas and Ho, 2005). In addition, FWS CWs require extensive land conversion for construction, which would be another disadvantage for use in developing nations' urban and slum areas.

A study done in Flanders comparing over 100 constructed wetlands in the area, showed that of the 54 FWS CWs in use, the average size was $7m^2$ per population equivalent (PE) (PE in this study was determined by the number of people connected to the system and not by the organic or hydraulic loading rates) (Rousseau et al., 2004). Whereas, the average size for VFCWs was 3.8 m^2 PE^{-1} (Rousseau et al., 2004). A comparison study of horizontal flow CWs in Denmark showed an average surface area demand of 5 m^2 PE^{-1} (Brix et al., 2007). Vertical flow constructed wetlands would have the smallest land use conversion demand, as well as a lower ecological impact, making VFCWs a more practical water treatment option for urban and slum areas. In addition, FWS CWs often fail to obtain the same removal efficiencies and nutrient conversions as can be obtained with VFCWs when treating high strength wastewaters such as dairy parlour washings, and concentrated animal feeding operation (CAFO) runoff. The anoxic environment found in the soils of FWS constructed wetlands means they are less efficient at nitrogen removal than vertical subsurface flow constructed wetlands (Kadlec and Scott, 2009). The Flanders comparison study showed an average 31% total nitrogen removal efficiency for the FWS CWs compared to 33% and 48% for horizontal and vertical subsurface flow constructed wetlands, respectively (Rousseau et al., 2004)

Free water surface CWs have also been shown to achieve lower removal efficiencies for pathogenic and indicator microorganisms than both subsurface horizontal and vertical flow constructed wetlands. Gerba et al. (1999) conducted a comparison study for indicator microorganism and pathogenic protozoa removal efficiencies in three different wastewater treatment systems including, a duckweed pond (aquatic ponds), a multi-species subsurface flow, and multi-species surface flow constructed wetland. Both wetlands were planted with *Thypha domingenisis*, *Scirpus olneyi*, *Salix nigra,* and *Populus fremontii.* The FWS CWs received flow from a small aquatic system containing water hyacinth. Samples of influent and effluent were collected from each system over a 34-month period, and subsequently analyzed for protozoan parasites, fecal coliform bacteria, turbidity, and pH. Average influent levels, for the aquatic ponds, of *Giardia* cysts and *Cryptosporidium* oocysts were 15.6 cysts l^{-1} and 1.58 oocysts l^{-1}, respectively. For fecal coliforms, the average influent density was 1.77×10^{6} CFU 100 ml^{-1}. Average effluent levels for *Giardia, Cryptosporidium*, and fecal coliforms were 0.35 cysts l^{-1}, 0.17 oocysts l^{-1}, and 5.97×10^{5} CFU 100 ml^{-1}, respectively. For FWS CWs, average influent densities for the same three microorganisms were 55.3 cysts l^{-1} for *Giardia*, 12.6 oocysts l^{-1} for *Cryptosporidium*, and 7.1×10^{6} CFU 100 ml^{-1} for fecal coliforms. Average concentrations in the effluent were 16.1 cysts l^{-1}, 4.9 oocysts l^{-1}, and 4×10^{4} CFU 100 ml^{-1} for *Giardia, Cryptosporidium*, and fecal coliforms, respectively. Finally, the subsurface flow wetland received influent levels of 62 cysts 100 l^{-1} for *Giardia*, 45.1 oocysts 100 l^{-1} for *Cryptosporidium*, and 1.2×10^{6} CFU 100 ml^{-1} for fecal coliforms.

Average concentrations in the effluent were 1.0 cyst 100 l^{-1}, 7.4 oocysts 100 l^{-1}, and 1.7 × 10^4 CFU 100 ml^{-1} for *Giardia*, *Cryptosporidium*, and fecal coliforms, respectively. The authors concluded that the subsurface flow constructed wetland had the highest removal for all indicator organisms (Gerba et al., 1999).

When considering both the decreased area demand, higher oxygen transfer rate of the vertical sub-surface flow (VSSF) constructed wetland, and higher overall removal efficiencies for nutrients and pathogenic bacteria, VSSF CWs may be better suited for wastewater treatment in developing nations rural and urban areas, as well as for higher strength agricultural wastewaters (Rousseau et al., 2004).

2.3.1. Subsurface Vertical Flow Constructed Wetlands Processes

The ability of constructed wetlands to utilize and transform wastewater constituents are driven by four main parameters, including soil/bed media, wetland plants, wetland microorganisms, and hydrologic condition (Moshiri, 1993). Each parameter is dependent upon the other, and functions efficiently when the relationships between the four parameters are optimized. In the following sections, the importance and function of each parameter will be discussed in further detail.

2.3.2. Subsurface Vertical Flow Constructed Wetland Bed Material

The soil, or bed medium, with which the constructed wetland has been built, performs several important functions within the wetland ecosystem such as, substrate for macrophyte growth, substrate for microorganism growth, and precipitation and adsorption sites for metals and chemicals in wastewaters (Novotny, 2003, Kadlec and Scott, 2009, Moshiri, 1993). The rooting media has three primary properties which dictate the interactions the media will have with the microorganisms, plants, metals and chemicals, which are porosity, specific surface area and chemical composition (Kadlec and Scott, 2009). Porosity has a significant impact on the hydraulics of a constructed wetland and will therefore be discussed in later sections. Specific surface plays an important role in supporting microorganism populations. High specific surface areas will support a higher bacterial population in a smaller volume (Kadlec and Scott, 2009). High specific surface area has also been linked to an increase in nitrification and biochemical oxygen demand (BOD) removal (Burgoon et al., 1991). The chemical composition of the medium used to construct the wetland plays a critical role in precipitation and adsorption of metals and chemicals (Kadlec and Scott, 2009).

Secondly, the capacity of a soil to adsorb and retain chemical components in a wastewater depends largely on the soil's chemical composition (Novotny, 2003). Organic matter composition and cation-exchange capacity plays a large role in adsorption. Cation-exchange capacity is the ability of a soil to adsorb and exchange positively charged ions (Novotny, 2003). Clays and organic matter are negatively charged, whereas several important wastewater constituents such as ammonia and heavy metals are positively charged. Once these cations adsorb to the negative soil surfaces, they are not readily released (Novonty, 2003). Typically, wetlands are constructed with sands and gravels for an optimal hydraulic conductivity (Kadlec and Scott, 2009). Finer materials and organic soils provide a greater surface area for microbial growth and unlike pure sands and gravels, contain an abundance of charged soil particles therefore have a higher cation-exchange capacity (Kadlec and Scott, 2009, Novotny, 2003). These materials have a disadvantage, however, of being of poorer hydraulic conductivity and are typically prone to clogging with biosolids (Hua et al., 2010, Langergraber et al., 2003). Sands and gravels, as a bed media, will accrete plant residuals and solids during a wetlands lifespan, and will have an increase in organic matter composition allowing for greater metal precipitation and chemical adsorption (Novotny, 2003, Moshiri, 1993, Stumm and Morgan, 1996). Newly constructed wetlands will often see a significant increase in removal efficiencies as the wetland ages (Kadlec and Scott, 2009).

2.3.3. Subsurface Vertical Flow Constructed Wetland Macrophytes

Wetland macrophytes are composed of four primary types, including emergent soft tissue plants, emergent woody plants, submersed aquatic plants, floating plants, and floating mats (Kadlec and Scott, 2009). Subsurface flow constructed wetlands will be planted with either emergent soft tissue plants such as *Scirpus* or *Carex spp*, or emergent woody plants such as *Salix* or *Betula spp* (Kadlec and Scott, 2009). Wetland macrophytes aid in wastewater treatment by incorporating nutrients and elements into plant tissue, and accretion of plant residuals, while the roots and stems provide surface for microorganism populations, facilitating oxygen transfer into soil through photosynthetic processes (Moshiri, 1993). Wetland macrophytes also provide carbon needed by functional heterotrophic bacteria which contribute to chemical processing of wastewater pollutants (Kadlec and Scott, 2009). Wetland plants create unique environments around the root zone, providing an oxygenated environment for microbes residing immediately around the root zone. Munch et al. (2007) found that the root surface of the wetland plant, *Glyceria maxima*, had an unexpectedly high level of microbial colonization, as opposed to an inert technical carrier material, which contained high levels of inactive zones. Despite the thinness of the rhizoplane biofilm on the plant roots, there were no inactive layers, indicating the importance of the root as a interactive bio-site for microbial growth and activity (Munch et al., 2007). Atkinson and Watson (2000) found that, in addition to plant root oxygen transfer to the plant rhizosphere, plant root exudates were able to oxidize compounds, toxic to the plant, around the plant rhizosphere. Microbial populations benefited not only from the aerobic environment but from the absence of toxic compounds (Atkinson and Watson, 2000). In addition, as plants grow and die, natural, stable residuals accrete in the wetland, providing areas for greater metal and chemical partitioning and adsorption (Kadlec and Scott, 2009, Moshiri, 1993). Plant growth, death and litter are also a significant nutrient sink within the wetland system (Kadlec and Scott, 2009).

Choosing a genetically diverse population of wetland macrophytes for a constructed wetland is critical for the living organisms in the wetland environment to utilize wastewater constituents and pollutants for their growth and reproduction, and to modify the quality of the water. Lack of diversity often results in unwanted species competition which requires high maintenance costs and can lead to a failed constructed wetland (Kadlec and Scott, 2009).

2.3.4. Subsurface Flow Constructed Wetland Microorganisms

The diversity of wetland microflora is immense. The microorganisms in a wetland ecology are the primary facilitators in the assimilation, transformation and recycling of chemicals, metals and nutrients (Moshiri, 1993). Microorganisms have the easiest access to the dissolved nutrients found in the pore water of the wetland bed media, and are the first to colonize any wetland system in great numbers (Kadlec and Scott, 2009). Fungi, bacteria and algae are the three main types of wetland microorganisms (Kadlec and Scott, 2009).

Fungi are eukaryotic organisms whose energy and carbon needs are met by the degradation of dead organic material. Because of their role in the degradation of dead material, they are the primary mediators in recycling nutrients, and make nitrogen, phosphorus and carbon more available for larger microorganisms. They live symbiotically with algae and plants, allowing their hosts to function more efficiently in the absorption of nutrients from the water, air and soil (Kadlec and Scott, 2009). Giraud et al. (2001) determined that *Absidia cylindrospora*, *Cladosporium sphaerospermum*, and *Ulocladium chartarum* fungal species played an active role in the removal of two polycyclic aromatic hydrocarbons: anthracene (AC) and fluoranthene (FA) within a constructed wetland. The authors determined that the fungal species may act synergistically with bacteria and plants within the wetland ecology to adsorb and degrade the two potent hydrocarbons, AC and FA (Giraud et al., 2001).

The bacterial populations in a wetland environment can be classified by their metabolic requirements. Most bacteria can be classified as heterotrophs, meaning they must obtain their nutrition and energy requirements from organic compounds (Kadlec and Scott, 2009). Autotrophic bacteria can synthesize organic compounds from an inorganic carbon source, such as carbon dioxide (CO_2), and can be further classified as photoautotrophs and chemoautotrophs (Rittman and McCarty, 2000). Photoautotrophic bacteria can synthesize organic compounds from CO_2, with light acting as an energy source (Kadlec and Scott, 2009). Chemoautotrophs obtain energy through the oxidation of reduced inorganic compounds, and can utilize CO_2 as their external carbon source (Rittman and McCarty, 2000). All bacteria can be classified as obligate aerobic, anaerobic, or facultative anaerobic bacteria (Rittman and McCarty, 2000). This classification is based on the preferred electron acceptor during respiration, of each bacterial species (Rittman and McCarty, 2000).

Aerobic respiration is the biochemical process by which an energy source is decomposed or oxidized in the presence of oxygen. This process provides energy for the bacteria species, and involves complete oxidation of an organic or mineral molecule (Rittman and McCarty, 2000). An electron transport chain, which is the driver for energy production, requires oxygen molecules to proceed (Rittman and McCarty, 2000). Some critical obligate aerobes within the wetland environment are the chemolithoautotrophs, *Nitrosomonas* and *Nitrobacter* (Kadlec and Scott, 2009). These species are critical consumers of various forms of nitrogen (Kadlec and Scott, 2009).

Anaerobic respiration occurs in the absence of oxygen with alternative electron acceptors such as sulfate, hydrogen sulfide, carbonate, and nitrate (Rittman and McCarty, 2000). Microorganisms whose metabolism is driven by anerobic respiration are responsible for important processes within wetlands, such as: reduction of nitrate, reduction of sulfate, reduction of iron, fermentation and methanogensis (Kadlec and Scott, 2009). There are a wide range of diverse, active anaerobes within a wetland ecology. A variety of anaerobic organotrophs including, *Pseudomonas, Alcaligenes, Bacillus, Agrobacterium, and Flavobacterium*, can reduce nitrogen (Kadlec and Scott, 2009). However, there are anaerobic chemolithotrophs such as *Thiobacillus*, and *Thiomicrospira* which can reduce nitrogen as well (Kadlec and Scott, 2009). The list of nitrate reducers also includes diazotrophs, which contain an iron-molybdenum nitrogenase system, and *Halobacterium*, which belong to the domain Archaea (Kadlec and Scott, 2009). *Desulfovibrio* bacteria are capable of reducing sulfate to hydrogen sulfide in the absence of oxygen (Kadlec and Scott, 2009). However, the conditions in most VFCWs do not facilitate the growth and activity of anaerobes. Flow conditions, which will be described in a later section, provide a constant source of oxygen into the wetland bed matrix, thereby prohibiting dissolved oxygen levels from reaching zero (Kadlec and Scott, 2009). Fermenation and methanogenesis occur at the bottom end of pε conditions, long after all the dissolved oxygen has been utilized as an electron acceptor (Kadlec and Scott, 2009, Rittman and McCarty, 2000, Stumm and Morgan, 1996). Therefore, the predominant bacteria found in VFCWs will most likely be limited to aerobic chemoheterotrophs, lithotrophs and facultative anaerobes (Kadlec and Scott, 2009)

The third most predominant microorganism in constructed wetlands is algae. Algae are often the earliest colonizers in a wetland and may be multi- or unicellular photosynthetic organisms (Kadlec and Scott, 2009). The two categories of algae are periphyton which grow on submerged substrates, and benthos which grow on the bottom of the water body (Vymazal, 1995). Algae found in a VFCW environment will be solely periphyton, as there is no permanent standing water with VFCW hydraulic patterns. Algae that grow attached to substrates in a wetland may be attached to four types of material, which are stones, mud or sand, plants, and animals (Kadlec and Scott, 2009). Epiphytons, or algae attached to plants, play an important role in the nutrient cycling between the algae community and the macrophyte to which they are attached (Kadlec and Scott, 2009). In addition, algae may play critical roles in metals remediation. Bender et al. (1994) studied the role that periphytic cyanobacteria had in reducing zinc and manganese concentrations in lab-scale packed columns. The cyanobacteria were grown in columns packed with glass wool as a substrate for adherence. The cyanobacteria achieved 96% zinc removal and 85% manganese removal from high concentration water (14mg l^{-1} of each metal). The authors concluded that because the concentrations of the metals were far higher than organism tolerance, the mechanism for removal could not be attributed solely to cell sequestration but to oxide precipitation as well (Bender et al., 1994). The authors posited that the pH levels, dissolved oxygen levels and the symbiotic presence of sulfate reducing bacteria, created an environment suitable to the formation of stable, non-toxic metal precipitates (Bender et al., 1994).

2.3.5. Subsurface Flow Constructed Wetland Hydrology

Wetland hydrology is defined as the study of the movement, distribution and quality of water within a wetland (Kadlec and Scott, 2009). The hydrologic conditions of a wetland control all other factors which influence the quality of water. The hydraulic conditions of the wetland system must meet the water needs of the wetland plants (Moshiri, 1993). The flow and storage volume controls the length of time the water has in contact with the wetland biota (Kadlec and Scott, 2009). The duration of the water-biota interaction, as well as the proximity of wastewater constituents to the biological, physical and chemical sites of activity will determine the extent of constituent alteration, and therefore the extent of the water remediation (Kadlec and Scott, 2009, Moshiri, 1993). In addition, maintaining the appropriate hydraulic conditions will necessarily maintain the correct water depth and flows which will inhibit the problems that can arise without the proper conditions, such as biosolids clogging (Kadlec and Scott, 2009).

Hua et al. (2010) reported that clogging in vertical flow constructed wetlands (VFCWs) is one of the most significant operational problems. According to the authors, clogging arises primarily from the accumulation of suspended solids and microbial biomass sedimentation which blocks the wetland bed pores. Clogging inhibits infiltration at a substantial rate after occurrence (Hua et al., 2010). Low infiltration rates inhibit the transport of oxygen and particulates through the wetland media, thereby reducing the rate of aerobic decomposition and reducing the contact between the wetland microorganisms and plants and wastewater constituents. This process results in a significant decline in wetland treatment performance (Hua et al., 2010).

In response to the issue of bed matrix clogging, Zhao et al. (2003) sought to develop a new bed matrix configuration that could potentially delay the development of clogging. These systems are labeled as anti-sized reed beds. The authors built two vertical flow reed bed systems with five different beds representing stages 1–5, according to the sequence through which wastewater passed. One set of five reed beds was built with progressively-sized bed matrices (size of particles increases from top to bottom layer) and the other built with anti-sized bed matrices (size of particles decreases from top to bottom layer). Each reed bed was made of Perspex columns, 900 mm in length and 95 mm in diameter, planted with *Phragmites australis*, and built with a 350 mm upper layer, 300 mm middle layer, and 150 mm supporting layer. In the progressively-sized media bed, gravel size was 4.4±1.5 mm for the upper and middle levels, and 26.4±7.2 mm for the bottom level. For the anti-sized media bed, gravel size was 9.8±2.7 mm for the upper level, 4.4±1.5 mm for the middle layer and 26.4±7.2 mm for the bottom layer. A diluted pig slurry influent was fed to the reed beds in a tidal flow pattern. Influent levels of suspended solids (SS) and BOD varied greatly from 133-1402 mg l^{-1} and 441-3150 mg l^{-1}, respectively. The authors determined that mean BOD, chemical oxygen demand (COD), NH_4-N, and phosphorus removal did not vary significantly between the two systems (Zhao et al., 2003). Suspended solids removal did not vary between the two systems either; however the authors noted that the anti-sized system received a much higher influent SS concentration during the trial period than did the progressively-sized system. The anti-sized reed bed received wastewater with an influent concentration of 740±312 mg SS l^{-1} versus 444±215 mg SS l^{-1} for the progressively-sized system (Zhao et al., 2003). Despite the higher SS influent load, the time to clogging for the anti-sized reed bed system was 17 d during the first stage and 43 d during the second stage. This is compared to a clogging time of 6 d for the progressively-sized system during stage one, and 30 d during stage two. Stage five showed the greatest difference in time to clogging for the two systems with 90 d and 285 d for progressively-sized and anti-sized systems, respectively. While clogging did occur in the first stage for both reed bed systems, the problem with clogging was considered more serious in the progressively-sized system, and occurred at a higher frequency. Clogging was delayed in the anti-sized reed bed as result of deeper effective SS filtration. This study and others also showed a marked decrease in removal efficiencies for ammonia-nitrogen and phosphorus after clogging occurrences (Zhao et al., 2003). After the clogging occurred in the progressively-sized reed bed, effluent ammoniacal-nitrogen increased from approximately 40 mg l^{-1} to 124 mg l^{-1}, with a coinciding effluent phosphorus concentration increase of approximately 20 mg l^{-1}(Zhao et al., 2003). These results indicate that clogging can have a large impact on removal efficiency. However, properly engineering SSF wetland hydraulics can decrease the occurrence of clogging (Kadlec and Scott, 2009).

Vertical flow constructed wetlands function under predominantly unsaturated flow conditions (Kadlec and Scott, 2009). Typically, VFCWs have low average hydraulic loading rates (HLR). Under a low average HLR, the wetland media remains unsaturated. The void spaces in the wetland bed matrix contain air at all times, unless the HLR exceeds the drainage rate (Kadlec and Scott, 2009). To maintain consistent aerated conditions within the bed matrix, the top layer of the wetland bed can be flooded. The flooding creates saturated conditions and potential surface ponding. The surface ponding creates an air lock as the air in the soil voids becomes trapped. Once this air-lock is broken, drainage through the bed matrix continues as unsaturated flow (Kadlec and Scott, 2009). Air continues to move into the pores from the atmosphere by diffusion and oxygen consumption by the wetland biota. The floodings or pulses can be repeated any number of times during a treatment dose, but should be timed in such a way that the previous dose of wastewater has percolated completely through the SSVF wetland bed matrix. SSVF wetlands can be rested for any period of time between these floodings, or pulses for enhanced aeration and organic matter decomposition (Kadlec and Scott, 2009).

2.4. Project Design

The objective of this research was to develop a constructed wetland treatment system which would be low-tech, economically viable, and easy to maintain. In designing the system, eight different vertical flow constructed wetland designs were compared for treatment effectiveness and low surface area requirements. The specifications of the eight VFCWs are detailed in the following section, and can be found in Table 2.1.

The study conducted by Morris and Herbert (1997) was the only VFCW, of the eight VFCWs compared, used to treat an agricultural wastewater. The sugar beet processing wastewater used in the study was characterized by elevated chemical oxygen demand (COD) and ammonia. Morris and Herbert (1997) reported adequate removal efficiencies for both COD and ammonia, however 143 m^2 of surface area was required to treat 3000 l d^{-1}. A surface area requirement of 143 m^2 prohibits portability, which was a specific objective of this research. Langergraber et al. (2010) reported better reductions in ammonia concentrations than Morris and Herbert (1997) while treating domestic sewage, however the hydraulic loading regime was much lower than that used by Morris and Herbert (1997). In addition, the surface area requirements for the two studies by Langergraber et al. (2010) required VFCWs sized at 10 m^2 and 20 m^2, which would also prohibit portability. The Garcia-Pérez et al. (2009) recirculating vertical flow constructed wetland, while achieving excellent removal efficiencies of BOD, fecal coliforms (FC), TSS, and ammonia, was also operated under a low hydraulic loading rate, with a 37.2 m^2 surface area requirement.

Noorvee et al. (2005) used a VFCW to treat dual-chamber septic tank outflow, and while the VFCW exhibited adequate removal efficiencies for BOD_7 (BOD measured after a 7 d incubation period), the VFCW failed to achieve nitrification levels typical for VFCWs (Kadlec and Scott, 2009, Noorvee et al., 2005).

Arias et al. (2010) constructed a two stage experimental treatment system for domestic sewage treatment, and although the VFCW used in the Arias et al. (2010) research was more compact than VFCWs presented by Morris and Herbert (1997) and Noorvee et al. (2005), the authors reported the lowest reduction in FC counts (Arias et al., 2003, Garcia-Pérez et al., 2009, Gross et al., 2007, Paulo et al., 2009). Paulo et al. (2009) constructed a VFCW in a 2.26 m^2 fiber-glass tank, which would have facilitated portability, however the authors reported lower reductions in BOD, FC, TSS, and total phosphorus (TP), than the VFCW constructed by Gross et al. (2007), which had the smallest surface area requirement operated with the second highest hydraulic loading regime.

2.4.1. Model Design

Of the eight constructed wetland designs, the system designed by Gross et al. (2007) appeared to have the attributes that best fit the objectives of this research (See Table 2.1). This RVFCW had the greatest ratio of HLR to wetland surface area requirements, and achieved the best removal efficiencies for the selected target contaminants, including BOD, fecal coliform, TSS, ammonia, and TP. Gross et al. (2007) constructed a RVFCW as a greywater treatment system for a single family household in Israel. In addition, the authors designed three different operation study modes, including a short-term study, a batch greenhouse study, and a long term case study. The basic wetland design consisted of a three tank system, with a primary sedimentation tank which received greywater with an average influent BOD level of 466 mg l^{-1} and a TSS concentration of 158 mg l^{-1}. The water then flowed to the root zone of a CW unit planted with *Cyperus haspan* and *Hydrocotyle leucocephala*. The wetland unit was composed of three layers, with a 15 cm thick top layer composed of planted organic soil, over a 30 cm layer of tuff or plastic media, draining through a 5 cm layer of limestone pebbles. The bottom of the wetland bed compartment was perforated, so water that trickled through the filter bed could flow to a reservoir and then be pumped again to the root zone. The size of the field study RVFCW was approximately 1 m^2, with a depth of 0.55 m. The design volume was based on the daily average greywater volume produced from a family of five in Israel. The authors designed the wetland volume from the Kadlec and Knight (1996) suggested model:

$$A = \frac{Q}{k} \; x \; Ln \; \frac{(C_i - C^*)}{(C_e - C^*)} \tag{2.1}$$

Where A is the surface area of the wetland (m^2), Q is the water flow rate (m^3 d^{-1}), C_e is the outlet target concentration of the design parameter, C_i (mg L^{-1}) is the inlet concentration of the design parameter, C^* (mg L^{-1}) is the background concentration of the design parameter, and k (mg d $^{-1}$) is the first-order areal rate constant of the design parameter. The authors designed the wetland based on a BOD loading rate, with a desired outlet concentration of 10 mg l^{-1}. The five-member family household used for the study produced a greywater flow of approximately 450 l d^{-1}, for a HLR of 0.45 m d^{-1}. The batch greenhouse and the long term case study showed mean removal efficiencies between 90% and 99% after a 12 h recirculation time for BOD, TSS and total nitrogen (TN). Total nitrogen and phosphorus were reduced by 75% and 60% respectively after 8 h of recirculation time. Fecal coliform removal varied greatly between each study stage. In the short term study, with an influent fecal coliform count ranging from 9×10^4 – 1×10^8 CFU 100 ml^{-1}, the count dropped by three to four orders of magnitude after 8 h, and reached 10 CFU 100 ml^{-1} after a recirculation time of 88 h. During the batch greenhouse study however, with an average influent FC count of 5×10^7 CFU 100 ml^{-1}, the count dropped by only two orders of magnitude. The variation in fecal coliform removal efficiencies during the trial exceeded the Israeli standards for unlimited greywater reuse, at times. The results of this study implies that the potential for improving FC removal exists.

The primary difference between the systems developed by Gross et al. (2007) and the comparison CWs, is the media used in the wetland. Traditionally, constructed wetland bed matrices are composed of several layers of gradated sand and gravel material, starting with fine grained sands as a top layer. The lower layers are composed of gradually larger diameter particles. The abrupt particle size change from the first layer to the second layer was done to optimize high surface area, which facilitates enhanced treatment, while maintaining a relatively fast flow rate. The goal of this research was to find a constructed wetland system that would be suitable for both domestic greywater and higher strength agricultural waters. The general approach for this research was based on the premise that the Gross et al. (2007) design better facilitated both types of wastewater, as the organic soil layer would adsorb greater amounts of phosphate, a nutrient found in high levels in agricultural wastewater (Novotny, 2003). In addition, the greater surface area of the organic soil layer would provide a larger interface for biota-contaminant interaction, and therefore greater reduction of target contaminants.

2.4.2. Portability Assessment

The design presented by Gross et al. (2007), in comparison with the seven other constructed wetlands assessed, also had the most potential for portability. The CW has a surface area requirement of 1 m^2, and had the greatest ratio of HLR to surface area (See Table 2.1). The design also incorporated a recirculating flow component, the benefits of which are described below. However, the primary attribute of the Gross et al. (2007) design which facilitates portability is the above ground design. The CW unit was built in a plastic container, sitting on top of but not connected to a plastic container serving as the reservoir. The units could easily be removed from the treatment location and transported to areas in need of emergency wastewater treatment in the event of a hydrologic disaster, which may destroy municipal wastewater treatment systems.

2.4.3. Recirculated Vertical Flow Constructed Wetlands

The recycled flow pattern was also adopted for this research due to the evidence of greater removal efficiencies found by Gross et al. (2007) and Sklarz et al. (2010). The principle behind recirculating flow back into the wetland substrate is to continue to circulate wastewater through the treatment wetland until the desired effluent quality is achieved. Sklarz et al. (2010) reported that previous research done in Israel by the same authors, showed that an additional 6 hr recirculation period was sufficient to bring the quality of treated domestic wastewater into compliance with Israeli regulations for irrigation use. The authors also found that the removal rates of target contaminants correlated exponentially with the recirculation flow rate, and that the optimal recirculation flow rate was estimated at 1.5 m^3 h^{-1}. As both greywater and agricultural water was going to be applied to the VFCWs for this research, a system was needed with mechanisms for improving treatment capacity within the range of influent wastewater quality. Recirculating flow would provide that mechanism for increasing the treatment capabilities of the same wetland as the unit transitioned from relatively low strength greywater to high strength dairy wastewater.

2.5. Cost Analysis

2.5.1. Life Cycle Assessment

The cost and energy requirements involved in developing wastewater treatment systems are accrued in two places: capital and operation. Capital costs can be divided into three parts: materials, transport and process (Dixon et al., 2003). The main cost of constructed wetlands is due to the materials required for construction. Gravels and sands are not only the most expensive part of a constructed wetland, but in many geographical locations, are considered non-renewable resources (Dallas and Ho, 2005). In addition, the weight and bulk of gravels and sands inherently add additional transportation costs that may not occur during the transport of materials needed for other wastewater treatment options such as: aerated filtration and activated sludge processes. According to a lifecycle assessment study performed by Dixon et al. (2003), in which the energy requirements for a constructed wetland was compared to an aerated biological filter (ABF) sized for 12 population equivalents (PE), the amount of energy, in megajoules (MJ), required for obtaining materials for the CW was 16,771 versus 4,807 for the ABF. The transportation energy requirements for the CW was 9,433 MJ compared to 1,733 MJ for the ABF system. However, the operational energy needs for the CW was only 37% of the operational energy needs for the ABF system. The ABF system had a 46,001 MJ operational energy requirement, versus 17,280 MJ for the CW system. The total energy requirements for the ABF and CW system were 52,560 MJ and 43,621 MJ, respectively. Despite the much lower operational energy requirement for the constructed wetland, the two systems were comparable in overall energy requirements, due to the fact that the materials and transport energy cost for the CW was nearly 75% higher than the ABF (Dixon et al., 2003).

Similarly, Machado et al. (2007) reported that the fossil fuel requirement for a constructed wetland sized for 1 PE was 952 g versus 98 g and 38 g for slow rate infiltration and activated sludge, respectively. However, the electricity required for operating a constructed wetland was reported as negligible, compared to 321.2 kilowatt hours (kWH) per 1 PE for activated sludge process (Machado et al., 2007).

Gross et al. (2008) reported that the cost of irrigating a 150 m^2 family garden in the Negev desert, in Israel was approximately 30 US$ per month, and that the average monthly water consumption for the garden would be 23 m^3. If 45% of the family's greywater was dedicated to irrigating the garden, the family would attain a gross savings of 25 US$ per month. The authors reported that the return of investment (ROI) for a vertical flow constructed wetland providing pre-treatment for greywater irrigation, would be approximately 3 years.

Clearly, with such low operational energy requirements, constructed wetlands are a viable, long term wastewater treatment system for developing nations and decentralized rural communities. However, many impoverished communities simply do not have the capital required to implement a constructed wetland. A solution then, is to find alternative ways of building constructed wetlands to mitigate the capital costs inherent with using gravels and sands as wetland bed media.

2.5.3. Recycled Materials for Wetland Bed Material

Although there has been research in utilizing recycled waste or by-product materials in constructed wetlands, the research done has been almost solely oriented around enhancing phosphorus adsorption. Chazarenc et al. (2007) and Weber et al. (2007) both successfully used electric arc furnace steel slag columns located at constructed wetland outlets to enhance phosphorus removal. Zhao et al. (2008) used dewatered alum sludge cakes (DASC), a by-product of potable water treatment, as a constructed wetland substrate and reported a 42% phosphorus removal attributed solely to the DASC. The potential for replacing traditional media in trickling filters with recycled materials was detailed by Elliot (2001) and Mondal and Warith (2008). Elliot (2001) reported on the use of recycled glass fragments, in place of sand, in a recirculating granular media filter in an Oswego, New York wastewater collection system. The author reported that the recycled crush glass met the NYS-DEC [New York State Department of Environmental Conservation] discharge permit requirements for BOD, TSS, and ammonia. The recycled crushed glass also cost $12.50 per ton less than did traditional sand. The crushed glass also proved to be more durable than natural sand when subjected to a magnesium sulfate soundness and acid solubility test. The author concluded that overall, the economically and mechanically superior material was the recycled glass.

Mondal and Warith (2008) studied the efficacy of recycled rubber tire chips as a material for a trickling bed filter treating landfill leachate. The authors found that the trickling filter was a successful biological treatment system, with BOD removal percentages between 81-96%.

Despite these studies, research on the use of alternative and viable wetland substrates, including PET plastic, to replace gravel is still incomplete at this time. Worldwide, approximately 2.5×10^{11} PET plastic bottles are consumed every year, and less than 3% of these bottles are recovered (CRI, 2008, Frigione, 2010). If this abundant material can be utilized effectively as a wetland bed material, material and transport energy requirements, and the costs associated with those energy requirements for vertical flow constructed wetlands would substantially decrease.

Dallas and Ho (2005) conducted a study in Costa Rica, Central America to determine if polyethylene terephthalate (PET) plastics, used as the primary bed media for a subsurface flow reed bed, would perform the same as a wetland constructed with traditional bed media, such as sands and gravels. The authors stated that in the United States, the cost of imported gravel for bed construction in artificial wetlands was between 40-55% of the total cost. In this study, the authors built four different reed beds in triplicate. The beds were filled with either two types of media, conventionally crushed rock or PET plastic drinking water bottle segments, and were either planted or unplanted. The macrophyte used in the planted reed beds was *Coix lacryma-jobi*. Due to the relatively small volume (75 l) of each, the total influent flow of greywater was no more than 5 l d^{-1} during Costa Rica's dry season and 10 l d^{-1} during the wet season. The authors reported that the PET plant reed beds showed higher reduction of BOD and fecal coliform concentrations than all other wetland types. Explanations made for higher fecal coliform removal was that the higher root mass found in the PET plant system increased available biofilm. The PET based reed beds showed an average wet plant biomass increase of 38 kg per reed bed, versus the 13.5 kg wet biomass increase for gravel based reed beds. The majority of the plant biomass was root mass, which was much higher for the PET beds (Dallas and Ho, 2005). The root zone is the most active area in a constructed wetland, providing surface area for microbial biofilms (Munch et al., 2007). Biofilms transfer oxygen and release exudates into the root zone. These two vital processes are responsible for molecular degradation of pollutants and nutrients (Kadlec and Scott, 2009, Munch et al., 2007). In addition, the hydraulic retention time was higher in PET plant reed beds due to the higher porosity of the PET plastic (Dallas and Ho, 2005).

Subsequent research on the use of PET plastic as a bed media is limited, and the Dallas and Ho (2005) paper failed to report on removal rates for other critical water quality parameters, which would help determine PET plastic performance in constructed wetlands. A particular oversight in the Dallas and Ho (2005) paper is the failure to analyze phosphorus removal efficiencies. Phosphorus is rarely found in dissolved form (particularly inorganic phosphorus), and is primarily adsorbed to sediment and soil particles (Novotny, 2003). Phosphorus movement primarily occurs as a result of three mechanisms, including plant uptake, reducing conditions, and adsorption (Novotny, 2003). For a constructed wetland, this means that the largest fraction of phosphorus present in the system is contained in the wetland soils and sediments (Kadlec and Scott, 2009). Eventually the bed matrix of a constructed wetland will reach the CWs assimilative capacity for phosphorus. The excessive accumulation can subsequently result in a precipitous decline in phosphorus retention, or result in sudden release in an event that has been termed as a chemical time bomb (Novotny, 2003). Phosphorus retention can decline sharply after 4-5 years of operation in a constructed wetland (Chazarenc et al., 2007). Combining a PET plastic bed matrix with a top layer composed of organic soil, such as the Gross et al. (2007) RVFCW design, may restore the phosphorus adsorption mechanisms not found in a PET plastic CW.

A long term study is needed to determine the true performance of a PET CW. If CWs built with PET plastic can achieve removal efficiencies comparable to treatment wetlands built with gravels and sands, and with a comparable life cycle, the potential for implementation of PET constructed wetlands in developing nations, and impoverished decentralized communities, greatly expands. Polyethylene terephthalate plastic is ubiquitous and often times entirely free in the form of millions of discarded drinking bottles in landfills. Constructed wetlands, which are already more economically viable for developing nations than traditional wastewater treatment facilities, could become a primary wastewater treatment system in nations without land-use conversion capabilities or gravel and sand resources (Denny, 1997, Wood et al., 2007).

2.6. Project Objective

The purpose of this research was two-fold, and was to duplicate the RVFCW system built by Gross et al. (2007) and to create a RVFCW system with PET plastic as the primary wetland bed media. The primary difference between the study presented by Gross et al. (2007) and the study presented for this thesis work, is the use of agricultural wastewater for treatment efficacy analysis, and the macrophytes used by the authors. The authors planted the RVFCW unit with *Cyperus haspan* (dwarf papyrus) and *Hydrocotyle leucocephala* (pennywort), neither of which was determined to be an appropriate macrophyte for this research. Pennywort is not native to North America, therefore was not chosen. Although dwarf papyrus is native to North America, the plant has accelerated reproduction, can disperse in unpredictable environments, has rapid nutrient uptake, and high peak biomass (Cronk and Fennessey, 2001). These characteristics make dwarf papyrus a highly invasive species, and in some geographical locations, a weed. In a wetland with 1 m^2 surface, a dominantly invasive species such as dwarf papyrus would out compete other beneficial plant species. The plant species planted in the RVFCWs used for this research was *Juncus balticus* (Baltic rush), *Carex nebrascensis* (Nebraska sedge), and *Scirpus americanus*, (three-square bulrush).

Baltic rush is a perennial, rhizomatous wetland plant found in dry intermountain regions of North America, and stands 30-90 cm tall (NRCS-USDA, 2010). In addition, Baltic rush has a dense root mass which makes the plant an excellent choice for high flow velocities (NRCS-USDA, 2010). Dense root masses help prevent erosive effects of higher loading rates, and water velocities (Kadlec and Scott, 2009, Novotny, 2003).

Nebraska sedge is a perennial, heavily rhizomatous wetland plant, growing typically 90 cm tall. The plant has high tolerance for alkaline conditions, and a deep, dense root system. Nebraska sedge also has high biomass, making the plant an excellent nutrient sink (NRCS-USDA, 2010).

Three-square bulrush is a tall plant, and can reach heights up to 150 cm (EPA, 2010). Three-square bulrush is highly resistance to flood conditions, with an anoxia endurance greater than 28 d (Cronk and Fennessy, 2001). Recommendations are to plant *Juncus* and *Carex spp* as companion plants to the three-square bulrush (EPA, 2010).

The three plant species were chosen for their dispersal rates, and their salt tolerance. In addition, the wetland plant ecology performs more efficiently with species of different heights, therefore a short, medium height, and tall plant species was chosen for this research (D. Cooper, personal communication, January 6, 2009).

2.7. Summary

In summary, constructed wetlands are a valuable tool for wastewater treatment. Constructed wetlands are the most economically and environmentally sustainable wastewater treatment systems available to developing countries at this time. However, some design modifications need to be made in an effort to make constructed wetlands more affordable for impoverished or decentralized communities and agricultural operations. Using recyclable materials, in place of traditional gravels and sands as the primary wetland bed material, is the first step in developing a more affordable CW. However, current research on the efficacy of recycled plastics in a CW is incomplete, and should be investigated further.

For many of the benefits already outlined, RVFCWs are the wetland system of choice for investigation into a sustainable, holistic CW design. Recycled vertical flow constructed wetlands would be much easier to incorporate within urban and slum areas due to the decreased surface area required for implementation (Rousseau et al. 2004). In addition, inherent within the characteristics of the construction model, VCFWs are easier to build with recirculation flow capabilities than horizontal flow or surface flow constructed wetlands, as less piping and diversion construction material would be required to return flow to the CW inlet. Recycled vertical flow constructed wetlands, which can recycle effluent until a certain effluent quality is achieved for reuse as irrigation or bathing water, will be critical components in the ongoing challenge to find water sources in water scarce regions. In the face of the UN's renewed commitment to the world's children, who suffer most from poor water quality, constructed wetlands may be the most feasible method to achieve some of the more critical water related Millennium Development Goals without relying on financial aid from industrialized nations (Denny, 1997, United Nations, 2008).

Appendix A

Table 2.1: Comparison of constructed wetland surface area requirements, type of media used, hydraulic loading rates, and various target contaminants for previously studied vertical flow construction wetlands.

References	CW* Area, m^2	Media Type	HLR†, $m\ d^{-1}$	Percent Removal of Target Contaminants BOD‡	FC§	TSS¶	Ammonia	TP**
Gross et al. (2007)	1	layer 1 - 0.15 m organic soil layer 2 - 0.3 m volcanic tuff layer 3- 0.05 m limestone pebbles	0.45	100%	2 log units	98%	NR	71%
Morris and Herbert(1997)	143	Cell 1 - 0.65 m coarse sand Cell 2 - 0.7 m fine sand	0.139	50%	NR	58%	74%	NR
Arias et al. (2003)	5	layer 1 - 0.8 m 0-2 mm gravel layer 2 - .2 m 8-16 mm gravel	0.945	NR	1.7 log units	NR	NR	NR
Noorvee et al. (2005)	37.4	limestone layers	NR	66%	NR	NR	22%	22%
Garcia-Perez et al. (2009)	37.2	layer 1 - 0.61 m 4 mm gravel layer 2 - .61 m 13-25 mm stone	0.048	99%	2 log units	98%	96%	33%
Langergraber et al. (2010)	10	0.5 m 2-3.2 mm gravel	0.07	99.10%	NR	NR	99.89%	20.50%
Langergraber et al. (2010)	20	Cell 1 - 0.5 m 2-3.2 mm gravel Cell 2 - 0.5 m 0.06 - 4 mm gravel	0.07	98.60%	NR	NR	99.60%	31.10%
Paulo et al. (2009)	2.26	layer 1 - 0.05 m fine gravel layer 2 - 0.55 m coarse sand layer 3 - 0.1 m fine gravel layer 4 - 0.2 m coarse gravel	0.22	86%	86%	81%	60%	55%

Chapter 3

Evaluation of a Portable Recycled Vertical Flow Constructed Wetland as a Treatment System for Greywater Reuse.

3.1 Abstract

The purpose of this study was to develop and evaluate a portable, recycled vertical flow constructed wetland (RVFCW) with a low surface area requirement, and low capital construction costs, which would achieve biologically acceptable contaminant removal efficiencies during the treatment of domestic greywater. In addition, the research objective was to evaluate the treatment efficacy of RVFCW units constructed with recyclable PET plastic, to determine if the PET plastic could viably replace traditional gravel as the primary bed media. Nine water quality monitoring parameters were chosen to determine RVFCW contaminant and nutrient removal capabilities, including total phosphate (TP), ammonia-nitrogen, nitrate-nitrogen, sulfate, total suspended solids (TSS), total dissolved solids (TDS), total organic carbon (TOC), total plate count (TPC), and fecal coliforms (FC). The RVFCW units showed a 185±178% increase ($p = 0.0014$) in TP concentrations, a 78.6±26% ($p < 0.0001$) decrease in ammonia-nitrogen, and a 5887±2992% ($p < 0.0001$) increase in nitrate-nitrogen concentrations. Total suspended

solids (TSS) and TDS concentrations increased by 26±139% ($p = 0.0036$) and 320±185% ($p < 0.0001$), respectively. The units achieved a 52±19% decrease ($p < 0.0001$) in TOC concentrations during GW treatment. pH values increased by 21±1.6% ($p < 0.0001$) during treatment. The units achieved a 2 log reduction ($p < 0.0001$) in TPC and a 3 log reduction ($p < 0.0001$) in FC, during treatment. Sulfate values were considered invalid, due to contamination of the RVFCW units by water used to water the units during the pre-trial, growth period, which was characterized by high sulfate concentrations.

The two types of RVFCW units performed differently for three of the parameters, including ammonia-nitrogen, nitrate-nitrogen and pH. The RVFCW units constructed with PET showed a 72.8±33% decrease ($p = 0.0323$) in ammonia concentrations, which was 16.2% less than the ammonia reduction achieved by the RVFCW VT units. Conversely, the RVFCW PET units showed a 4742±2526% increase ($p = 0.0155$) in nitrate-nitrogen concentrations, which was 48.2% less than the effluent nitrate concentrations of the RVFCW VT units. The RVFCW PET unit pH values were 10.9% less ($p = 0.0132$) than the pH values observed for the RVFCW VT units, during GW treatment.

These results indicate that the RVFCW units are adequate primary treatment mechanisms for bacterial contamination in greywater. However, under current design parameters, the RVFCWs are not entirely suitable as a holistic treatment system for greywater if nutrient and solids reduction is a priority. In addition, the elevated RVFCW unit effluent nitrate-nitrogen concentrations require an additional treatment step if nitrate reduction is a treatment goal. The results of this study also indicate that PET plastic can be successfully used as a construction medium without compromising treatment efficacy.

Design modifications should be made and evaluated before the RVFCW units can meet human health safety standards for unlimited reuse as irrigation water, and for overall improvement of contaminant removal efficiencies.

3.2. Introduction

Greywater is domestically generated water, excluding water generated from toilets. Greywater is predominately water generated from laundry, bathing, and washing dishes, and accounts for 50-75% of household water consumption (Maimon et al., 2010, Paulo et al., 2009). Increasing human populations in water stressed areas are driving the need for greywater recycling and reuse, particularly on-site reuse (Finley et al., 2009, Maimon et al., 2010, Nair, 2008, Travis et al., 2010). Maimon et al. (2010) performed a critical review of guidelines for greywater reuse for irrigation, citing that the primary concerns with greywater reuse was exposure to water borne pathogens through direct contact or through consumption of contaminated plants, and exposure to peripheral vectors such as mosquitoes. In addition, the elevated levels of salts, boron, oils and surfactants may impair soil quality and contaminate groundwater. The authors suggested that due to the hazards associated with exposure, unlimited reuse of untreated greywater was not always recommended, even for single households. Travis et al. (2010) conducted a 40 d irrigation trial, comparing various contaminant levels of predominant greywater pollutants in sand, loess and loam. The authors reported that fecal coliform count reached as high as 100 CFU g^{-1} soil (in loam). Finley et al. (2009) reported a fecal streptococcus count of 71.52 CFU g^{-1} lettuce crop irrigated with untreated greywater, which was associated with a 20% higher risk of illness than ingesting lettuce crop irrigated with tap water. Clearly, greywater reuse without a form of primary treatment can pose serious risk to human health, as evidenced by a 2004 WHO report, which stated that, annually, exposure to unclean water causes the deaths of 1.6 million children under the age of 5 (WHO, 2004).

Constructed wetlands have provided a low-cost, low-technology, sustainable treatment system for greywater reuse in many industrialized and developing countries. Gross et al. (2007) developed a recycled vertical flow constructed wetland which successfully treated greywater for reuse of a single family household. The authors reported a 2 log reduction of fecal coliforms (FC), and reduced TSS, BOD, and COD by 98%, 100% and 81%, respectively. The constructed wetlands were able to treat greywater to a water quality level which met Isreali irrigation guidelines for all the above parameters but fecal coliforms. However, with 2 log reduction reported by Gross et al. (2007) and 3 log reduction of FC reported by Dallas and Ho (2005), constructed wetlands could provide a critical primary treatment stage in decentralized, impoverished and slum areas in need of greywater treatment and reuse.

One main impediment to implementing constructed wetlands is the initial capital cost associated with the harvesting and transportation of gravel needed to construct the wetland bed. The only clear way of reducing capital construction costs of constructed wetlands is by replacing gravels and sands as the traditional wetland bed media. Although recyclable materials such as glass and tire chips have been used in trickling filters, little research has been done using recyclable materials in constructed wetlands (Elliot, 2001, Mondal and Warith, 2008). Dallas and Ho (2005) utilized PET plastic as a primary wetland bed media instead of gravel, and reported a 3 log reduction of FC, and a 98% BOD reduction. However, research on PET as a wetland bed media is incomplete. Therefore, one objective of this research was to provide further insight into the viability of replacing traditional gravels and sands with PET plastic as the primary bed media in treatment wetlands, and to determine if PET plastic would affect removal rates of wastewater contaminants.

3.3. Materials and Methods

3.3.1 Recycled Vertical Flow Constructed Wetland Unit and Reservoir Construction

Unit Body

Four 1.16 m^2 recirculating vertical flow constructed wetland (RVFCW) units were built to fulfill the objective of this research. The body of the wetland unit was composed of a modified 1.22 m diameter galvanized aluminum livestock watering tank. The bottom of the tank was removed and a standard, expanded, ¾ in, plain steel grating was welded in place. The grating was treated with a cold steel galvanizing, zinc based paint. The modified tank was set on a 1.23 m tall, plain steel, six-legged stand, which stood inside another 1.22 m diameter galvanized aluminum livestock watering tank. The top tank was labeled as the RVFCW unit, and the bottom tank was labeled as the reservoir. The six legged stand was coated with a polyurethane elastomer. This design was replicated for all four RVFCW units.

Tank modifications and stand construction took place at the Engineering Research Center at Colorado State University. The sedimentation basins, stands, and RVFCW units are pictured in Figure 3.1.

Bed Matrix

Two of the four units were built as follows, starting with the bed materials at the bottom: 5 cm of 70 - 75 mm coarse gravel, 30 cm of 25 - 30 mm volcanic tuff, 10 cm of 60% topsoil and 40% dairy compost soil. The RVFCW units built with this bed matrix were labeled as RVFCW VT units. The other two units were built as follows, starting with the bed materials at the bottom: 5 cm of 70-75 mm coarse gravel, 20 cm of polyethylene terephthalate plastic pieces cut approximately 7-10 cm long, 15 cm of 60% topsoil and 40% dairy compost soil. These RVFCW units built with this bed matrix were labeled RVFCW PET units.

Wetland Macrophytes

The wetland macrophytes used in this research were bought from Aquatic and Wetland Company (Fort Lupton, Colorado). The plants used were *Carex nebrascensis* (Nebraskan sedge), *Juncus balticus* (Baltic rush), and *Scirpus americanus* (three-square bulrush). Eight plants of each species were planted in the 7 – 15 cm thick organic soil layer, for a total of 24 plants per RVFCW unit. Prior to planting, the soil layer was heavily watered to the point of saturation. A 15 cm × 6 cm hole was dug with a trowel, and the seedling was placed in the hole. The mulch was filled back in around the seedling, with a small mound built up around the base of the plant. The soil was left loosely packed around the roots. All plants were immediately watered after planting.

Lighting

High intensity fluorescent lighting was provided for the RVFCW units to help initiate growth indoors during the winter months. Pioneer (Sunleaves® Garden Products, Bloomington, IN) 4 Bulb Grow lights, with 54-watt T5 Bloom tubes, were chosen as the grow lights. The grow lights burned at 2900° K color temperature and were kept on for 12 hr a day. The lights were turned off and on automatically by a timer. The lights were hung approximately 65 cm above the top of the RVFCW unit tank, by a stand constructed with 2 in PVC pipe (Figure 3.1).

3.3.2. Sedimentation Basins

Wastewaters flowed into the RVFCW units from 2.43 m diameter galvanized aluminum livestock watering tanks. These tanks were labeled as SEDBASINS. The sedimentation basins were placed on top of 2.13 m tall, six-legged plain steel stands coated in epoxy acrylic paint (Figure 3.1). These sedimentation basins were modified with two, 2 in diameter steel female threaded outlets, welded 8 in from the bottom, on the outside of the tank, 0.9144 m apart. Each one of the outlets provided flow to one of each type of RVFCW unit. For example, SEDBASIN 1 provided flow to RVFCW PET Unit 4 and RVFCW VT Unit 2. SEDBASIN 2 provided flow to RVFCW PET Unit 1 and RVFCW VT Unit 3.

3.3.3. Flow Distribution and Subsurface Irrigation System

The wastewaters were distributed evenly throughout the RVFCW unit by a subsurface irrigation system fed by a 2 in polyvinyl chloride (PVC) pipe connected by a ball valve at each of the sedimentation basin steel threaded outlets. The subsurface irrigation system was built with four way tee junctions, with each of the radial joints connected to sections of ¾ in diameter PVC pipes, drilled with 1/8 in drill bits at 1 in intervals on the downward side of the pipe. Each section was capped with a ¾ in diameter PVC slip cap. The length of the pipe sections at the first and fifth junctions was 0.3175 m, the length at the second and fifth junctions was 0.4064 m, and the length of the sections connected to the third junction was 0.4572 m. The pipes were then buried at a depth of 5 cm in the traditional RVFCW units, and a depth of 8 cm in the RVFCW PET units.

Recirculation flow was provided to the subsurface irrigation system by an EasyPro® (Pittsburg, CA), 200 gallon per hour (gph) submersible magnetic drive pump.

3.3.4. Wastewater Source, Transport, and Storage

The greywater used for the first phase of this research was obtained from one dormitory shower block at Colorado State University. The greywater from the shower block flowed into a 300 gal storage tank which could be accessed from an outside faucet. The greywater was pumped into a 450 gal transportation tank and taken to the RVFCW location, where the greywater was pumped into a 1500 gal on-site storage tank.

3.3.5 Study Design

Dosing Patterns

A minimum of 24 hr prior to dosing the RVFCW units with 350 l of the greywater, the 2.43 m diameter sedimentation tanks were filled with greywater stored in the 1500 gal on-site storage tank. On each dose day, 350 l of water was released from the sedimentation basins onto each of the RVFCW units attached to the tank. During the first phase of the study, the ball valves were fully opened, and 45 l was fed to an RVFCW unit. When surface ponding had entirely disappeared, another 45 l was fed to the RVFCW unit. This was repeated until 350 l had passed through each RVFCW unit. After the initial dose of 350 l was completed, the 200 gph submersible pond pumps were turned on for 16 hr to recirculate the water from the unit reservoir back to the subsurface irrigation system in the RVFCW unit. Dosing took place on six dates from April to June, 2010 when the RVFCW units were the following ages: 56 d, 75 d, 106 d, 120 d, 127 d, 134 d.

Hydraulics

The average hydraulic retention time (HRT) and hydraulic loading rate (HLR) for all four RVFCW units was 0.152±0.087 d^{-1} and 0.37±0.178 m d^{-1}. The average recirculation rate was 5.72±2.8 m^3 d^{-1}. Hydraulic loading rate and hydraulic retention time calculation methods are described in the following paragraph.

Each 45 l dose was measured by a ruler, tracking the drop in water level in the SEDBASIN, and timed from the moment the ball valve was opened to the moment surface ponding ceased. When the HLR calculations were performed, the first pulse time period and volume were ignored assuming that after the first pulse of greywater had passed through the RVFCW unit, the topsoil was saturated. Therefore, the assumption would be that flow through the topsoil layer would be a steady-state flow. In addition, the short and rapid flow-path through the high-porosity middle matrix would also create a steady-state flow pattern (Kadlec and Wallace, 2010, Sklarz et al., 2010). If these two assumptions are made, HRT and HLR could be calculated with the following equations (Kadlec and Scott, 2009):

$$Q = \frac{V - 45.42\,l}{T - t} \tag{3.1}$$

Where Q is water flow rate ($m^3\ d^{-1}$), V is the total volume dosed (m^3), which was calculated by measuring the depth of water in the RVFCW reservoir and multiplying that value by the surface area of the reservoir (0.371 m^2) , T is the total time measured for the entire dose (d), and t is the time measured for the first pulse (d). HLR was calculated with the following equation (Kadlec and Scott, 2009):

$$q = \frac{Q}{A} \tag{3.2}$$

Where q is HLR (m d^{-1}), and A is the RVFCW unit surface area (m^2). HRT was calculated with the following equation (Kadlec and Scott, 2009):

$$\tau = \frac{V_{active}}{Q} \tag{3.3}$$

Where τ is HRT (d^{-1}), V_{active} is the volume of the RVFCW unit containing water in active flow (m^3), and Q is water flow rate ($m^3\ d^{-1}$).

Sampling Method

Water sampling was conducted at four different locations within the wastewater treatment flow stream: source (at the dormitory directly from the shower block water reservoir), sedimentation basin outflow, RVFCW unit outflow, and recirculation outflow. At each sampling time, eight bottles of sample water were taken for analyses of fecal coliforms (FC), total plate count (TPC), nitrate-nitrogen (NO_3^-- N), ammonia-nitrogen

(NH_3 - N), sulfate, total phosphate (TP), total organic carbon (TOC), total suspended solids (TSS), total dissolved solids (TDS), and pH. Separate acid washed bottles were used for the analyses of TP, NO_3^- - N, and NH_3 - N, and the water samples used for these analyses were immediately acidified to a pH less than 2 with 1M hydrosulfuric acid for preservation. Each bottle was placed into a cooler with ice packs for transportation. The water samples were stored at 4°C until analyzed.

3.3.6. Sample Analyses

Colorimetric Procedures

Total Phosphate

TP was analyzed by the EPA approved HACH® (Loveland, CO) method 8190, with persulfate digestion. The water samples were collected as previously described in section 3.3.5. Prior to analysis for TP, the samples were neutralized with 5M sodium hydroxide (NaOH) solution to a pH between 7.1 and 7.5. After neutralization, 5 ml of sample was added to a Test N' Tube (Hach Co., Loveland, CO) vial, followed by a packet of potassium persulfate powder. The tube was inverted 15 times and then placed in a COD reactor set at 150° C, for 30 min. After this time, the Test N' Tubes were removed from the reactor and allowed to cool to room temperature. After the tubes were cooled, 2 ml of 1.54 N NaOH solution was added to the tube. The tube was capped and inverted ten times, wiped with a moist paper towel, followed by a lint free tissue and read in a spectrophotometer at a wavelength (λ) of 890 nm. The instrument was zeroed at this value and the tube removed from the instrument. A PhosVer 3® (Hach Co.) powder pillow was added to the Test N' Tube. The tube was capped, inverted 10 times, and left to react for 2 min. After 2 min, the tube was wiped clean, and read again. A three point calibration curve was created, with total phosphate standards, at the beginning of each set of analyses with the concentration values 2.5 mg l^{-1}, 5 mg l^{-1}, and 10 mg l^{-1}. The concentration of TP for the water sample was calculated by the equation derived from the calibration curve and the sample's absorbency value.

Nitrate-Nitrogen

Nitrate-nitrogen was analyzed by EPA approved HACH® method 10020, with chromotropic acid. Prior to analysis for nitrate, the samples were neutralized with 5M sodium hydroxide (NaOH) solution to a pH between 7.1 and 7.5. After neutralization, 1 ml of sample water was added to a Nitrate Pretreatment Solution Test N'Tube (Hach Co.). The tube was inverted 10 times, wiped clean with a wet paper towel and a lint free tissue, then placed in a spectrophotometer, and read at a wavelength of 410 nm. The instrument was zeroed at this value, and the tube removed from the spectrophotometer. A NitraVer X Reagent B® (Hach Co.) powder packet was added to the tube. The tube was inverted 10 times to mix, then allowed to react for 5 min. After 5 min, the tube was wiped clean, and read again. A three point calibration curve was created, with nitrate standards, at the beginning of each set of analyses with the concentration values 2.5 mg l^{-1}, 5 mg l^{-1}, and 10 mg l^{-1}. The concentration of nitrate-nitrogen for the water sample was calculated by the equation derived from the calibration curve and the sample's absorbency value.

Ammonia-Nitrogen

Ammonia-nitrogen was analyzed by the EPA approved HACH® salicylate method 10031. Prior to analysis for ammonia, the samples were neutralized with 5M sodium hydroxide (NaOH) solution to a pH between 7.1 and 7.5. After neutralization, 100 µl of de-ionized water and 100 µl of sample water were added to two separate AmVer Reagent Diluent® (Hach Co.) vials, with the de-ionized water acting as the sample blank. The contents of one Ammonia Cyanurate (Hach Co.) powder packet and one Ammonia Salicylate (Hach Co.) powder packet was added to each vial. The vials were inverted 15 times, and then allowed to react for 20 min. After 20 min, the vials were wiped clean with a wet paper towel, followed by a lint free tissue, then placed in a spectrophotometer and read at a wavelength of 655 nm. The instrument was initially zeroed with the de-ionized water vial. A three point calibration curve was created, with ammonia standards, at the beginning of each set of analyses with the concentration values 2.5 mg l^{-1}, 5 mg l^{-1}, and 10 mg l^{-1}. The concentration of ammonia-nitrogen for the water sample was calculated by the equation derived from the calibration curve and the sample's absorbency value.

Sulfate

Sulfate was analyzed with the EPA approved HACH® method 8051. The water samples were stored at 4°C prior to analyses and allowed to warm to room temperature. 10 ml of de-ionized water and 10 ml of sample water were added to two separate HACH DR 2500 spectrophotometer sample cells. The contents of one SulfaVer 4® reagent powder packet was added to each sample cell. The sample cell was swirled vigorously until all the powder was dissolved After a 5 min reaction time, the sample cell was wiped clean with a wet paper towel, followed by a lint free tissue, then placed into a spectrophotomer to be read at a wavelength of 450 nm. The instrument was initially zeroed with the de-ionized water sample cell. A three point calibration curve was created, with sulfate standards, at the beginning of each set of analyses with the concentration values 10 mg l^{-1}, 20 mg l^{-1}, and 30 mg l^{-1}. The concentration of sulfate for the water sample was calculated by the equation derived from the calibration curve and the sample's absorbency value.

Total Iron

Total iron (TI) was periodically measured at random times and RVFCW units to ensure that iron levels never reached concentrations which would interfere with the colorimetric processes used to measure: ammonia, nitrate, and total phosphate.

An Aquachek® (Hach Co.) total iron (0 – 5 mg l^{-1}) kit was used to determine TI concentration. A water sample was taken from a random RVFCW reservoir, from either the RVFCW outflow or the recirculation outflow, during the duration of the trial. The sample vial was filled half full with sample water, and an Iron Reducing Powder pillow (Hach Co.) was added to the vial. The vial was shaken rapidly for 5 s. The vial was opened and a dip test strip was placed in the sample water and moved rapidly back and forth for 15 s, then the dip test strip was removed and excess water was shaken from the end, before the test pad was compared to the color chart.

Gravimetric Procedures

Total Suspended Solids

Total suspended solids (TSS) were determined using an adaptation of Method 2540 from the 21st edition of Standard Methods for Examination of Water and Wastewater (Eaton et al., 2005). The water samples were stored at 4°C until date of analyses, and then warmed to room temperature. A Millipore® (Billerica, MA) 47 mm diameter, binder-free, glass fiber filter, with 1µm porosity, was desiccated in a convection oven at 110°C for 1 hr. At the end of 1 hr, the filter was removed from the oven and cooled in a desiccator to room temperature. The filter was weighed, and then placed into a 250 ml capacity, Nalgene® (Thermo Fisher Scientific, Rochester, NY) polysulfone filter cartridge holder with receiver. The sample bottle was inverted 10 times to mix, then 250 ml of water sample was measured out in a glass graduated cylinder. The filter cartridge was connected to an air flow manifold, and a vacuum pump was turned on to create suction, to pull the wastewater through the filter. The filter was removed from the filter holder after all 250 ml had passed through into the receiver, and then placed in a pre-dried and pre-weighed aluminum weigh dish. The filter was dried in a convection oven at 110°C for 12 hr. At the end of the drying period, the filter was removed and placed in a desiccator to cool to room temperature, and weighed again. The concentration of TSS was calculated with the following equation:

$$\text{mg l}^{-1} \text{ of total suspended solids} = \frac{(A-B)\ x\ 1000}{Sample\ Volume, ml} \tag{3.4}$$

Where A is the weight of the filter + dried residue (mg), and B is the weight of the filter (mg).

Total Dissolved Solids

Total dissolved solids (TDS) were determined using an adaptation of Method 2540 from the 21st edition of Standard Methods for Examination of Water and Wastewater (Eaton et al., 2005). Total dissolved solids was measured in conjunction with TSS, and followed the same protocol as described in the previous section, with the exception that the filtrate captured in the receiver of the filter holder was poured into a porcelain crucible previously dried for 1 hr at 110°C, and weighed. The crucible containing the filtrate of the water sample was placed back into the convection oven at 110°C, and dried for 12 hr, or until all the water had evaporated. At the end of the drying period, the crucible was removed and placed in a desiccator to cool to room temperature, and weighed again. The concentration of TDS was calculated with the following equation:

$$\text{mg } l^{-1} \text{ of total dissolved solids} = \frac{(A-B)\ x\ 1000}{Sample\ Volume, ml} \tag{3.5}$$

Where A is the weight of the crucible + dried residue (mg), and B is the weight of the crucible (mg).

High Temperature Combustion

Total Organic Carbon

Total organic carbon was analyzed using an adaption of Method 5310 from the 21st edition of Standard Methods for Examination of Water and Wastewater (Eaton et al., 2005). Samples were analyzed with a Shimadzu® (Columbia, MD) TOC-VCSH instrument, utilizing high temperature combustion with a nondispersive infrared sensor (NDIR). Samples were stored at 4°C until time of analyses. The samples were warmed to room temperature, and inverted 10 times to mix, before approximately 30 ml of water sample was poured into a Shimadzu® ASI-V auto-sampler sample cell. The instrument auto-calibrated with internal software, with total carbon (TC) standards of 50 mg l^{-1} and 100 mg l^{-1}, and inorganic carbon (IC) standards of 10 mg l^{-1} and 50 mg l^{-1}.

Microbial Analyses

Total Plate Count

Total plate count was performed with Petrifilm® (3M™, St. Paul, MN) Aerobic Plates. Samples were stored at 4°C, in sterile polypropylene bottles until time of analyses. Water samples were stored for no longer than 24 hrs from time of sampling to time of analyses. At time of analyses, the samples were removed from refrigerated storage and gently inverted 10 times. For each sample, a 10-fold dilution series was prepared (in lambda buffer (for 1 l of solution: 5.8 g NaCl, 2.0 g $MgSO_4 \cdot 7H_2O$, 50 ml 1 M Tris-HCl (Sigma-Aldrich®, St. Louis, MO) (pH 7.8), 0.1 g gelatin (Difco® Laboratories Inc, Franklin Lakes, NJ)) in the range of $10^{-1} – 10^{-5}$. After the serial dilution was complete, 1 ml of each dilution was placed onto a Petrifilm® Aerobic Plate, according to the manufacturer's instructions. The plates were incubated at 30°C for 48 hrs. At the end of the incubation period, the plates were removed, and total colony forming units were counted.

Fecal Coliforms

Fecal coliform counts were performed with Petrifilm® Coliform Plates (3M™). Each sample was diluted as described above, and 1 ml of dilution was placed onto a Petrifilm® Coliform Plate, according to the manufacturer's instruction. The plates were incubated at 44°C for 24 hrs. At the end of the incubation period, the plates were removed, and total colony forming units were counted.

pH

pH was measured with an Oakton® (Vernon Hills, IL) waterproof EcoTestr pH 2 pocket pH tester. The pH meter was calibrated with standards of pH 4, 7, and 10 prior to each day's measurements. The pH meter was rinsed with deionized water between each reading, and then wiped clean with lint free dry wipes and stored.

Statistical Analysis

Experimental data was analyzed using SAS® 9.2 software (SAS Inst. Cary, NC). An ANOVA mixed effects model was used, with degree of freedom approximated by the Kenward-Roger method. A significance level of $\alpha = 0.05$ was used to determine treatment effect. Treatment effects were divided into primary and secondary classes. Primary treatment effects were defined as the change in parameter values through the process of RVFCW unit action. Secondary treatment effects were defined as the difference in the parameter values as a result of the RVFCW unit types, PET and volcanic tuff (VT).

3.4 Results

3.4.1. Ammonia-Nitrogen

Ammonia is a widely used indicator of the efficacy of wastewater treatment systems (Kadlec and Scott, 2009). The production of ammonia is the first step in mineralization of organic nitrogen. In terms of wastewater quality, elevated ammonia-nitrogen concentrations may indicate high organic compound contamination associated with the aerobic and anaerobic processing of dead and dying cells and tissues (Kadlec and Scott, 2009). Therefore, the reduction of ammonia concentrations in constructed wetland effluent implies that organic nitrogen is being converted, through microorganism facilitated transformation, to various species of inorganic nitrogen, such as ammonium ion, nitrite, and nitrate.

The RVFCW units achieved excellent removal efficiencies of ammonia-nitrogen, with overall concentrations decreasing by 78.6±26% ($p < 0.0001$) during treatment (See Table 3.1). No significant variations were seen in any particular RVFCW unit, or on any particular sampling day. The dormitory shower water ammonia-nitrogen concentrations were steady throughout the entire 78 d sampling period (4.97±2.09 mg l^{-1}). As a result, the RVFCW units were not subject to large variation in ammonia loading rates throughout the trial period, which can impact the treatment efficiency of an artificial wetland (Kadlec and Scott, 2009). Ammonia concentration was one of the few water quality parameters affected by the difference in RVFCW unit media (See Figure 3.2). The PET plastic units removed a statistically significant ($p = 0.0323$) smaller percentage of ammonia-nitrogen than did the units constructed with volcanic tuff (See Table 3.2).

3.4.2. Nitrate-Nitrogen

Nitrate-nitrogen, in excess, can be a potent water pollutant. Elevated nitrate-nitrogen levels can lead to eutrophication of surface waters, as well as pose a human health risk (Novotny, 2003). The EPA sets a nitrate-nitrogen level of 10 mg l^{-1} for drinking water (Novotny, 2003). Greywater typically contains very low concentrations of nitrate-nitrogen. The Gross et al. (2007) study reported levels of 3.0±1.3 mg NO_3^- l^{-1}, but this study showed levels no higher than 0.08±0.09 mg NO_3^- l^{-1}. However, the dormitory greywater did not contain kitchen or laundry wastewater as did the greywater used in the

Gross et al. (2007) study. The addition of kitchen and laundry wastewater to a greywater waste stream will result in elevated nitrate concentrations (Friedler, 2004).

The RVFCW units showed an increase in nitrate-nitrogen concentrations through the treatment process (5887±2992%, $p < 0.0001$) (See Table 3.1). Again, there was no singular spike in nitrate-nitrogen concentrations at the source during the sampling period, nor did one single unit show highly elevated nitrate-nitrogen concentrations. Nitrate-nitrogen concentration was the second parameter impacted by the bed media type (See Figure 3.2). The RVFCW PET unit final nitrate-nitrogen outflow concentrations were measured at 3.82±1.99 mg l^{-1}, which was 47.3% lower than the final RVFCW VT unit outflow concentrations of nitrate-nitrogen (See Table 3.2).

3.4.3. Total Suspended Solids

For this study, a particulate in water which cannot pass through a pore size of 1µm was considered a total suspended solid. In greywater, TSS of this size will be composed of surfactants, oils, synthetic chemicals, protozoan cysts, bacteria, lint, hair, dusts, and grit (Free Drinking Water, 2011). In addition, many hydrophobic contaminants will adsorb to suspended solids. Therefore, elevated concentrations of TSS can be directly correlated to elevated concentrations of certain critical water contaminants. Gross et al. (2007) reported a TSS concentration of 158±30 mg l^{-1} in the raw greywater used in their research. The average TSS concentration found in the dormitory shower water was 29.6±3.25 mg l^{-1}. However, the greywater used in the Gross et al. (2007) study contained laundry and kitchen water, whereas the dormitory greywater was shower water only.

Total suspended solids were a highly variable measurement during the GW trial. The RVFCW units showed a slight overall increase in TSS concentrations by 26±139% ($p < 0.0001$). However, sedimentation accounted for 72±40% ($p = 0.0477$) decrease in TSS concentration from source concentrations. The RVFCW unit outflow accounted for a 865±885% ($p < 0.0001$) increase from sedimentation basin outflow concentrations, with recirculation accounting for another 21±125% increase ($p < 0.0001$) from the sedimentation basin outflow TSS concentrations (See Table 3.1). In addition, a small anomaly occurred on day 120, where average TSS concentration was elevated at all sampling locations, except the source. Total suspended solids were the only parameter which showed a unit effect. RVFCW PET Unit 1 TSS concentrations were statistically higher throughout the trial period, averaging 57.23±48.74 mg l^{-1}, or 81.7% higher ($p = 0.0218$) than the other three RVFCW units (See Figure 3.3). The elevated TSS concentration for RVFCW PET Unit 1 can be attributed entirely to recirculation outflow concentrations measured on 106 d and 120 d (See Figure 3.4). No secondary treatment effect was observed.

3.4.4. Total Dissolved Solids

Total dissolved solids (TDS) are another common water quality indicator. Dissolved solids can be composed of salts, metals, carbonate species, organic matter, and viruses (Free Drinking Water, 2011). The EPA sets a drinking water limit of 500 mg TDS l^{-1} (EPA, 2011).

The RVFCW units showed an increase in TDS of 320±185% ($p < 0.0001$), with no secondary treatment effect demonstrated (See Table 3.1). There was a large, statistically significant, spike in TDS concentrations in the RVFCW unit outflow and recirculation outflow on day 56 and 106. On both sampling days, a secondary treatment effect was seen, with the RVFCW VT Units showing overall higher TDS concentrations (See Figure 3.5).

3.4.5. Sulfate

Sulfate is a significant water quality parameter. When present in water, sulfate can be toxic to aquatic plants, animals, and fish. When present in irrigation, sulfate contributes to salinity levels as a major anion, leading to decreased crop yield (EPA, 2011).

Sulfate concentrations of the influent used to water the RVFCW units during the 60 d pre-trial period were measured at greater than 10,000 mg SO_4^{-2} l^{-1}. The RVFCW units subsequently displayed evidence of sulfate contamination during the trial period, with sulfate concentrations decreasing from an average of 442.50±149.97 mg SO_4^{-2} l^{-1} on day 56 to 81.41±11.32 mg l^{-1} on day 134 (See Figure 3.6). As the influent greywater sulfate concentrations were measured at 33±5.47 mg l^{-1}, well below the pre-trial influent water concentrations, any primary or secondary treatment effects were considered invalid for sulfate.

3.4.6. Total Phosphate

Total phosphate (TP) includes orthophosophate, inorganic phosphate, and organic phosphate (Kadlec and Scott, 2009). Total phosphate, in excess, is a critical water pollutant which causes algal blooms and eutrophication of surface waters (Novotny, 2003). The EPA indicates that phosphate levels should not exceed 0.05 mg l^{-1} in streams discharged into lakes or reservoirs (EPA, 1988). Gross et al. (2007) indicated greywater TP concentrations at 22.8±1.8 mg l^{-1}, however the greywater monitored for this study showed concentrations no higher than 1.45±2.05 mg l^{-1}. Again, the Edwards dormitory greywater was shower water only, and as indicated by Friedler (2004), wastewater from a washing machine contains much higher concentrations of phosphate than does shower water (169 mg PO_4^{-3} -P l^{-1} versus 10 mg l^{-1}).

The RVFCW units demonstrated an increase of TP concentrations by 185±178% ($p < 0.0005$) during GW treatment (See Table 3.1). However, during the course of the study, TP levels at each sampling point (source, SEDBASIN effluent, RVFCW effluent, and recirculation effluent) showed an overall decrease from the first sampling event to the last sampling event. The average TP values (averaged across all sampling points) observed were 7.02±3.46 mg l^{-1} on day 56 to 1.76±0.59 mg l^{-1} for on day 134 (See Figure 3.7). No secondary treatment effect occurred.

3.4.7. Total Plate Count and Fecal Coliforms

Total plate count (TPC) is the total number of viable, aerobic bacteria present in the sample. Total plate count is a very general indicator of water quality, but does not indicate the quantity of pathogenic organisms in the water (EPA, 2011). The bacteria counted in the TPC may include benign or beneficial bacteria as well as pathogenic bacteria (Kadlec and Scott, 2009). Fecal coliforms (FC) are a more specific indicator of water quality, and the concentration of FC in water does have known associations with outbreaks with certain diseases, though not as high an association as does *E. coli* or *enterococci* concentrations (EPA, 2011).

The RVFCW units were efficient at removing TPC, showing a 2 log reduction ($p < 0.0001$), with no significant change in TPC over time during the trial (See Table 3.1). There was no secondary effect observed during the trials.

The RVFCW units were also highly efficient at removing FC, showing a 3 log reduction ($p < 0.0001$) during the trial period (See Table 3.1). Again, there was no significant secondary treatment effect.

The source water remained relatively constant in both TPC and FC concentrations throughout the entire trial period, and no single RVFCW unit showed sudden spikes in TPC or FC concentration during the trial period, resulting in a constant TPC and FC loading rate.

3.4.8. Total Organic Carbon

Total organic carbon (TOC) accounts for both dissolved and suspended forms of carbon. TOC is an excellent water quality indictor as the presence of TOC in water is a product of the decomposition of plant, animal and microbial matter (Kadlec and Scott, 2009). Humic acids are soluble, large organic molecules which contribute significantly to the TOC concentration in waters, as well as to the color and odor of wastewater (Kadlec and Scott, 2009).

The RVFCW units achieved a 52±19% decrease ($p < 0.0001$) in TOC concentrations throughout the treatment (See Table 3.1). However, a notable trend was the 33±66% increase ($p < 0.0001$) in TOC concentrations from the sedimentation basin outflow to recirculation outflow (See Table 3.1). Sedimentation resulted in an initial 69±29% decrease ($p < 0.0001$) in TOC concentration.

No secondary treatment effect was observed.

3.4.9. pH

pH values for freshwaters should fall between 6.5 - 8 for optimal aquatic organism health (EPA, 2011). pH will also dictate the form and presence of anions, such as carbonate or sulfate, which can impact water and soil quality (Novotny, 2003).

pH levels of the dormitory greywater averaged 6.9±0.29 and rose at each level of treatment, to a final pH value of 8.3±0.10 (See Table 3.1). There was a secondary treatment effect observed with the RVFCW PET units demonstrating smaller increases ($p = 0.0132$) in pH during treatment (See Table 3.2).

3.4.10 Iron

Iron was analyzed periodically throughout the trial for the sole purpose of monitoring the levels to determine if the red coloration in the water from the metal grating in the RVFCW units would interfere with the colorimetric procedures used to measure ammonia, nitrate, and phosphate. The concentrations remained at 0.15 mg l^{-1} for total iron in the water collected from the RVFCW unit outflow and recirculation outflow for the duration of the trial. At this concentration, total iron concentrations did not interfere with the colorimetric procedures.

3.4.11. Hydraulics

No correlation was found between outlet concentrations of any of the parameters analyzed with either HRT or HLR. There was no statistically significant difference between the two RVFCW unit types and HRT or HLR values. There was no correlation between recirculation rate and recirculation treatment efficiencies.

3.5. Discussion

Vertical flow constructed wetlands have been used to treat greywater in a variety of scenarios and have been considered successful wastewater treatment systems (Kadlec and Scott, 2009, Rousseau et al., 2004). The necessity of finding ecologically and economically sustainable wastewater treatment systems has prompted a growth in the popularity of such wetlands to treat greywater for irrigation reuse. However, there are many irrigation water standards that must be met to protect human and environmental health (Maimon et al., 2010, Travis et al., 2010). Due to the population of people without access to any sanitation systems at all, priority must be made in finding a technology that can be implemented in impoverished communities without creating significant economic or ecological stress. The objective of this research was to develop a wastewater treatment technology that could treat water to biologically acceptable levels for wastewater recycling and reuse as irrigation water, flushing water, and even drinking water when combined with a low-cost, low-technology filtration step.

The secondary objective of this research was to find cheaper construction materials to replace traditional gravels and sands, in effort to reduce the initial capital cost of the artificial wetlands without compromising effluent water quality. The ubiquitous plastic, polyethylene terephthalate, was chosen as the alternative substrate for the RVFCW units. Nine wastewater quality parameters were chosen, for analysis, to determine the treatment efficacy of the RVFCW units used in this research. The analysis of the data obtained during the trial period would aid in the determination of the RVFCW units' potential as an effective wastewater treatment system, and if recycled PET plastic could effectively replace volcanic tuff.

3.5.1. Ammonia-Nitrogen

The primary mechanism for the reduction of ammonia-nitrogen in constructed wetlands is through nitrification (Kadlec and Scott, 2009). Nitrification begins with the breakdown of organic nitrogenous compounds, such as urea and amino acids, which produces ammonia (Kadlec and Scott, 2009). Ammonia reduction occurs when bacteria, such as *Nitrosomonas spp,* oxidize ammonia to nitrite in the presence of oxygen. The design of these RVFCW units created a highly oxygenated media, due to the abrupt change in media size from the top soil layer, to the middle volcanic tuff or PET layer, and the large grating sized used for the bottom of the RVFCW unit. Typically, VFCW design shunts effluent flow from the wetland through a single port, or outlet (Kadlec and Scott, 2009). With grating instead of a single outlet, the middle media layers never fully saturate with water. Also, the recirculation mechanism effectively re-oxygenates the RVFCW unit outflow, thereby providing another oxygen transport system to the RVFCW bed matrix, in addition to diffusion and convection.

The less efficient removal of ammonia-nitrogen seen in the RVFCW PET units was likely attributable to the high compression of the PET plastic. The VT rock is incompressible, but the PET compressed from an original depth of 42 cm to a final depth of 20 cm after the topsoil layer was added. The lack of, or smaller, pore space between the plastic strips would have inhibited the transfer of oxygen throughout the entire wetland media.

The concentrations of ammonia-nitrogen produced by both RVFCW unit types was higher than the taste and odor threshold concentration set by the EPA (0.2 mg l^{-1}), but is still significantly lower than the concentration levels found in some surface waters (12 mg l^{-1}) (EPA, 2001). Therefore, the difference in ammonia-nitrogen removal efficiencies found between RVFCW PET units and RVFCW VT units may be considered biologically insignificant in most wastewater treatment scenarios.

3.5.2. Nitrate-Nitrogen

Nitrate-nitrogen is a direct product of the bacteria mediated transformation of ammonia (Kadlec and Scott, 2009). Bothe et al. (2000) suggested that the oxidation of ammonia occurs in the following two steps:

$$NH_3 + O_2 + 2H^+ + 2e \xrightarrow{\text{Ammonia monooxygenase}} NH_2OH + H_2O \tag{3.6}$$

$$NH_2OH + H_2O \xrightarrow{\text{Hydroxylamine oxidoreductase}} NO_2^- + 5H^+ + 4e^- \tag{3.7}$$

A third step mediated by *Nitrobacter spp,* called nitrification, takes nitrite and oxidizes it to nitrate (Kadlec and Scott, 2009):

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- \tag{3.8}$$

Therefore, the decrease in ammonia-nitrogen concentrations during treatment would correspond directly to an increase in nitrate-nitrogen concentrations, as was seen during the trial period (See Figure 3.2). Nitrate concentrations can only be reduced through a process called denitrification, whereby nitrate is converted to dinitrogen (N_2) (Kadlec and Scott, 2009). This process occurs only under anaerobic conditions, and as previously stated saturated, anoxic flow conditions could never be obtained with the RVFCW design employed for this study.

The RVFCW PET unit outflow nitrate-nitrogen and ammonia-nitrogen concentrations were 3.14±1.81 mg NO_3^- - N l^{-1} and 3.08±1.98 mg NH_3-N l^{-1}, whereas the RVFCW VT unit outflow nitrate-nitrogen and ammonia-nitrogen concentrations were 4.83±2.92 mg l^- and 1.05±0.67 mg l^{-1}, respectively. The smaller difference in nitrate-nitrogen and ammonia-nitrogen concentrations in the RVFCW PET units indicates that anaerobic denitrification is occurring at a higher rate in the RVFCW PET units than in the RVFCW VT units (See Figure 3.2).

3.5.3. Solids

The increase in TSS concentrations observed during this research, is not consistent with previous studies done with constructed wetlands and TSS removal. Gross et al. (2007) and Garcia-Pérez et al. (2009) both reported 98% TSS removal rate with the RVFCWs used in their research. Paulo et al. (2009) reported a smaller 19% TSS removal achieved with a VFCW receiving water from a horizontal flow CW. The energy gradient of the water flow through the wetland media may have contributed to an erosive effect (Novotny, 2003). The soil particles from the RVFCW unit were effectively washed throughout the media and into the RVFCW reservoir. Recirculation also contributed to an increase in TSS concentrations, which could be attributed to recirculation rates exceeding the percolation rate, resulting in an overflow of the RVFCW unit. The recirculating water effectively bypassed the RVFCW unit, and flowed directly back into the reservoir.

Total dissolved solids are not a commonly reported parameter in constructed wetland research, and little data is available to support or conflict with the results of this research. However, the presence of dairy compost in the topsoil layer of the RVFCW unit may explain the large increase in TDS concentration through the treatment process. Dissolved solids are comprised of humic compounds, fulvic acids, and particulate metal species (Novotny, 2003). Ko et al. (2008) reported that animal waste compost is comprised of 1% fulvic acids, and 2.5-3% humic acids upon reaching composting maturity. In addition, the authors reported an overall increase in the concentrations of all the following heavy metals: copper (Cu), zinc (Zn), and lead (Pb). Therefore, the erosion seen in the topsoil layer throughout the trial period would contribute significantly to the TDS concentrations seen in the RVFCW effluent.

The TDS results observed during the GW trial indicates that topsoil with minimal or no dairy compost should be used as the top layer in RVFCW bed construction.

3.5.4. Total Phosphorus

Phosphate removal is difficult to achieve in constructed wetlands (Chaznarenc et al., 2007, Kadlec and Scott, 2009, Weber et al., 2007, Zhao et al., 2008). Phosphorus adsorption occurs in the presence of cationic soil components, and the majority of constructed wetlands are built with charge inert sands and gravels (Kadlec and Scott, 2009). Soils rich in clays and organics provide an optimal phosphorus adsorption capacity, which was the reason for incorporating an organic soil layer in the RVFCW design used in the study (Novotny, 2003). Although Garcia-Pérez et al. (2009) and Gross et al. (2007) both reported reductions in total phosphate concentrations (33% reduction for Garcia- Pérez et al. (2009) and 71% reduction for Gross et al. (2007)) during RVFCW greywater treatment, the RVFCW units used in this research demonstrated an increase in total phosphate concentrations throughout treatment. The increase in total phosphate observed in this study can be attributed to the same mechanism described for the increase in TSS and TDS concentrations. Animal manure is particularly high in phosphates, and the topsoil used in this study was composed of 40% dairy compost (Novotny, 2003). The presence of the RVFCW topsoil in the reservoir water, due to soil erosion, would increase TP levels.

3.5.5. Total Plate Count and Fecal Coliforms

Much data has been presented in support of the ability of constructed wetlands to remove bacteria from influent wastewater (Dallas and Ho, 2005, Gerba et al., 1999, Garcia-Pérez et al., 2009, Gross et al., 2008). The RVFCW units were no exception to these observations. Whereas Garcia-Pérez et al. (2009) and Gross et al. (2007) both reported a 2 log reduction in fecal coliform counts for RVFCW performance during the treatment of greywater, the RVFCW units used in this study achieved 3 log reduction in fecal coliforms. Although the effluent bacterial counts for both TPC and FC remained too high for many irrigation standards, potential design modifications or add-ons, could aid in further reduction of bacterial counts until effluent quality meets guidelines for greywater reuse as irrigation water (Maimon et al., 2010, Travis et al., 2010).

3.5.6. Total Organic Carbon

Most internal wetland processes rely on the organic carbon imports from influent wastewater. For example, the reduction of nitrate requires 3.02g of organic matter per gram of nitrate- nitrogen. In addition, a complex interaction between bacterial and plant respiration provides important sinks for influent carbon sources (Kadlec and Scott, 2009). The RVFCW units were very efficient at reducing TOC, as is typical for artificial wetland performance (Kadlec and Scott, 2009). The increase in TOC concentrations from the sedimentation basin outflow to the recirculation outflow is most likely attributable to the RVFCW TOC background levels.

3.5.7. pH

Kadlec and Scott (2009) report that the ability of artificial wetlands to buffer the pH variations and levels in wastewater, is due to the interaction between the water and wetland substrate, and not to any microbial activity. The addition of carbonate species from the sands, gravels, and soils would increase the alkalinity in the wetland substrate pore water, thereby increasing the buffering capacity of the wetland (Jensen, 2003). The lower pH value in the RVFCW PET units is most likely due to the inherent inertness of PET plastic, which cannot contribute to the alkalinity levels in the effluent. The difference in pH values between the RVFCW PET and RVFCW VT units is insufficient enough to contribute to unfavorable speciation of metals in the PET units, and therefore would not be considered a chemically significant effect from the PET plastic (Jensen, 2003).

3.6. Summary

The RVFCW units used in this study performed well for five of the nine parameters analyzed, including total organic carbon (TOC), total plate count (TPC), fecal coliforms (FC), ammonia, and pH. Design modifications need to be made to limit the amount of RVFCW unit topsoil erosion, which would help lower the effluent concentrations of TSS, TDS, and TP. The boundaries between media types should be composed of an intermediate media size, or should be composed of landscaping fabric, to prevent the loss of topsoil.

The increase in nitrate-nitrogen concentrations observed during treatment suggests that the RVFCW units should be used in series with an anaerobic wastewater treatment system, such as HSSF wetlands, if nitrate reduction is a primary treatment goal, or if influent nitrate-nitrogen concentrations are elevated (Kadlec and Scott, 2009).

Design modifications must be made and evaluated before these RVFCW units can be used as a stand-alone treatment system for greywater reuse as irrigation water. The RVFCW units, in their current configuration, produced outflow concentrations higher than the greywater influent concentrations in four of the eight critical water quality indicators (nitrate-nitrogen, total phosphate, total suspended solids, and total dissolved solids).

Overall, the RVFCW units show great promise as a sustainable wastewater treatment system and should be further modified to achieve better contaminate removal efficiencies. Also recommended, is the replacement of gravels and sands by PET plastic if the capital costs associated with harvesting and transporting gravels and sands could potentially prohibit the implementation of this wastewater treatment technology.

Appendix B

Figure 3.1: Detailed image of 1500 gallon water storage tank, sedimentation tanks, recycled vertical flow constructed wetland (RVFCW) units, RVFCW reservoirs, RVFCW grow lights, and effluent distribution pipes.

Table 3.1: Average values for target parameters measured at the four sampling points during greywater treatment, reported with percent removals after each treatment stage and total percent removal for the RVFCW treatment system. A decrease in parameter concentrations during greywater treatment is indicated by a positive percent removal, and an increase in parameter concentrations is indicated by a negative percent removal.

Parameter	Raw Shower Water Values*	Sedimentation Basin Outflow	% Removal	RVFCW† Unit Outflow	% Removal	Recirculation Outflow	% Removal	% Total Removal
Ammonia-N	4.97±2.09	5.31±2.12	+13±41	2.18±1.81	-60±23	1.06±1.36	-50±46	-78.6±26
Nitrate-N	0.08±0.09	0.72±0.44	+812±550	3.98±2.53	+837±1102	4.73±2.41	+27±28	+5887±2992
Total Phosphate	1.45±2.07	2.30±2.53	+59±171	3.58±2.92	+128±73	4.13±2.64	+76±188	+185±178
Total Organic Carbon	47.6±18.46	14.59±13.78	-69±29	18.28±6.15	+76±77	22.62±9.23	+33±66	-52±19
Total Suspended Solids	29.6±15.92	8.2±12.09	-72±40	30.6±11.96	+865±885	37.2±42.06	+21±125	+26±139
Total Dissolved Solids	141.2±30.51	252.67±37.08	+79±26	514.83±282.56	+98±86	593.08±267.46	+20±17	+320±185
Total Plate Count	$4.9\times10^7\pm8\times10^7$	$9\times10^5\pm1.6\times10^6$	- 2 log	$1.1\times10^6\pm2.8\times10^6$	+ 1 log	$5.2\times10^5\pm1.1\times10^6$	- 1 log	- 2 log
Fecal Coliform	$1.3\times10^7\pm1.4\times10^7$	$9.9\times10^4\pm2.2\times10^5$	- 3 log	$1.1\times10^5\pm2.9\times10^5$	+ 1 log	$3.5\times10^4\pm8.3\times10^4$	- 1 log	- 3 log
pH	6.85±0.30	7.8±0.31	+13.8±5	8.23±0.23	+5±3.8	8.3±0.20	+0.7±2.7	+21±1.6

* All values are reported as mg l^{-1}, except for total plate count and fecal coliforms which are reported as CFU ml^{-1}, and pH.
†Recycled vertical flow constructed wetlands

Table 3.2: Comparison of ammonia, nitrate, and pH levels, measured during greywater treatment trial, between the recycled vertical flow constructed wetland (RVFCW) units constructed with polyethylene terephthalate plastic as the primary wetland bed media and the RVFCW units constructed with volcanic tuff as the primary bed media. A decrease in parameter concentrations during greywater treatment is indicated by a positive percent removal, and an increase in parameter is indicated by a negative percent removal.

Parameter	Raw Shower Water*	Sedimentation Basin Outflow	RVFCW† PET‡ Unit Outflow	RVFCW VT§ Unit Outflow	RVFCW PET Recirculation Outflow	RVFCW VT Recirculation Outflow	RVFCW PET Unit Total Removal %	RVFCW VT Unit Total Removal %
Ammonia-Nitrogen	4.97±2.09	5.31±2.12	3.08±1.98	1.05±0.67	1.27±1.64	0.72±0.99	- 72.8±33	- 84.5±20
Nitrate-Nitrogen	0.08±0.09	0.72±0.44	3.14±1.81	4.83±2.92	3.82±1.99	5.63±2.53	+4742±2526	+7032±3207
pH	6.85±0.30	7.8±0.31	8.06±0.15	8.35±0.16	8.23±0.2	8.38±0.19	+ 20.1±2.8	+ 22.3±2.8

* All values reported in mg l^{-1}, except pH.
† Recycled vertical flow constructed wetland
‡ Polyethylene terephthalate
§Volcanic tuff

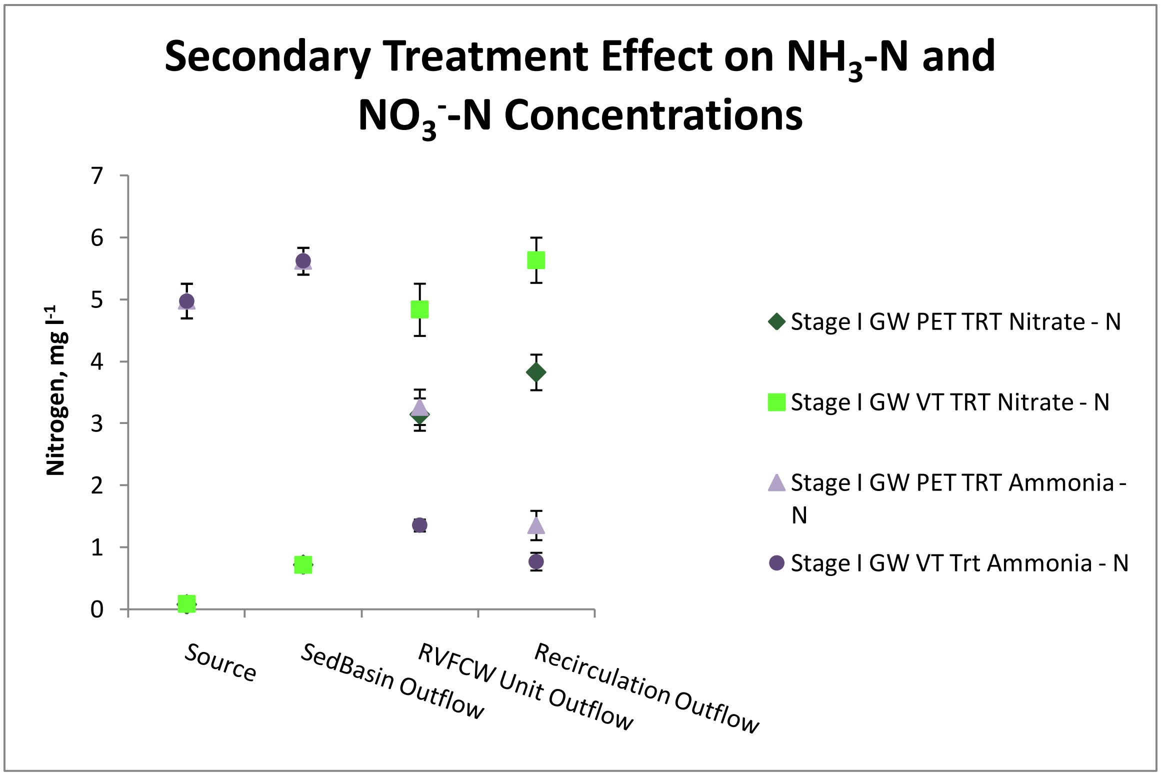

Figure 3.2: Comparison of recycled vertical flow constructed wetland unit type and concentrations of ammonia-nitrogen and nitrate-nitrogen, with standard error, at the sampling locations source, Sedbasin outflow, RVFCW unit outflow and recirculation outflow, during greywater treatment.

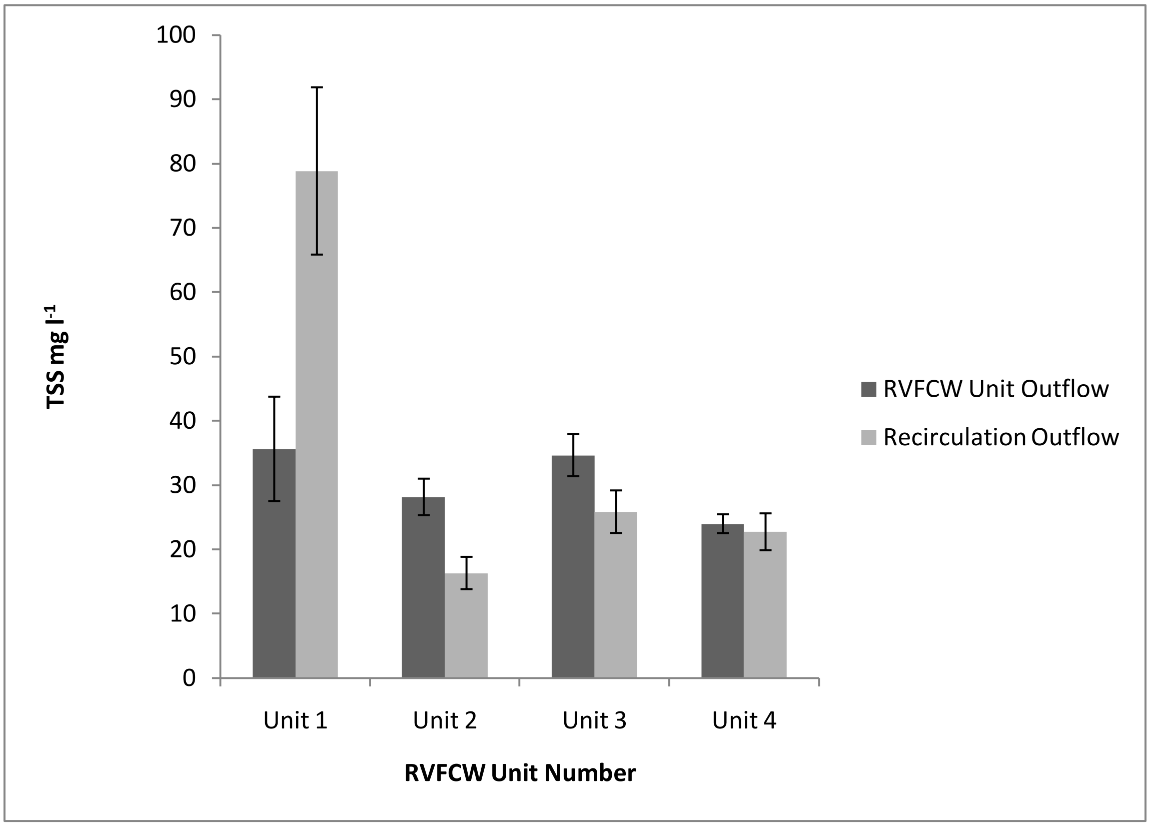

Figure 3.3: Recycled vertical flow constructed wetland (RVFCW) unit total suspended solids concentrations with standard errors, from the sampling locations RVFCW unit outflow, and recirculation outflow, during greywater treatment.

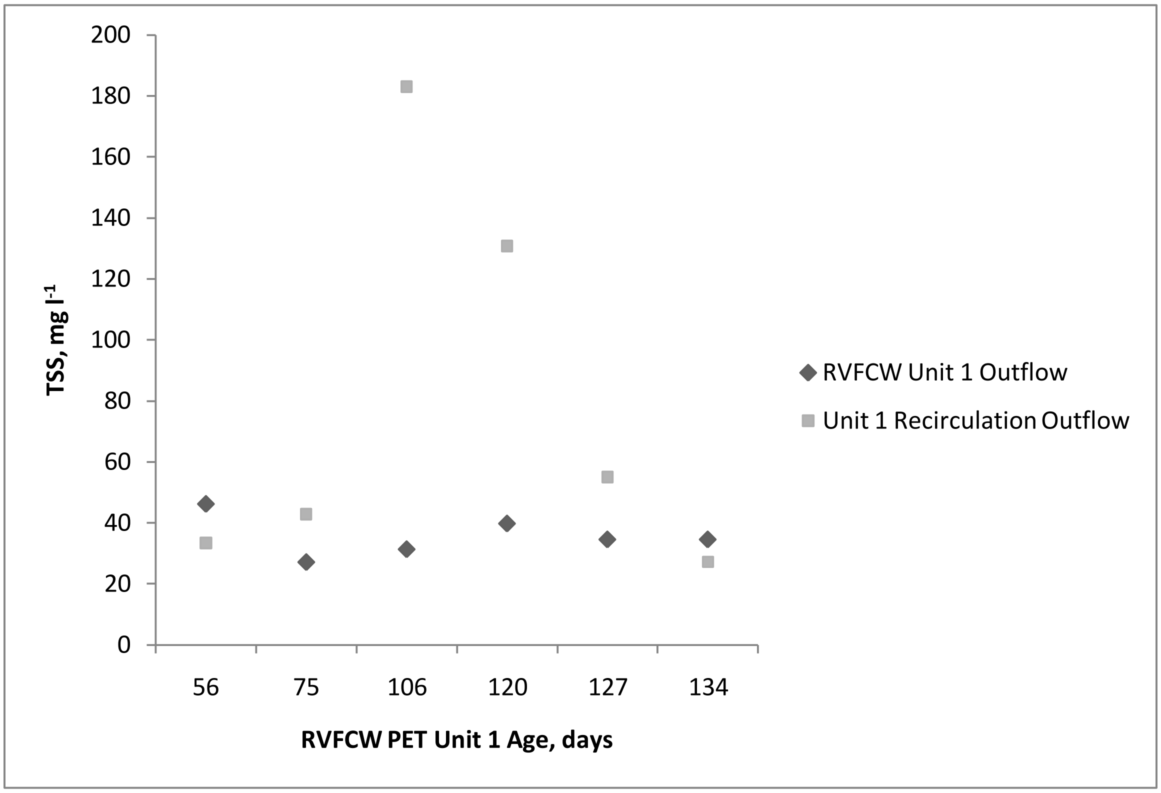

Figure 3.4: Recycled vertical flow constructed wetland (RVFCW) polyethylene terephthalate (PET) Unit 1 total suspended solids concentrations in RVFCW unit 1 outflow, and recirculation outflow, over time, during greywater treatment.

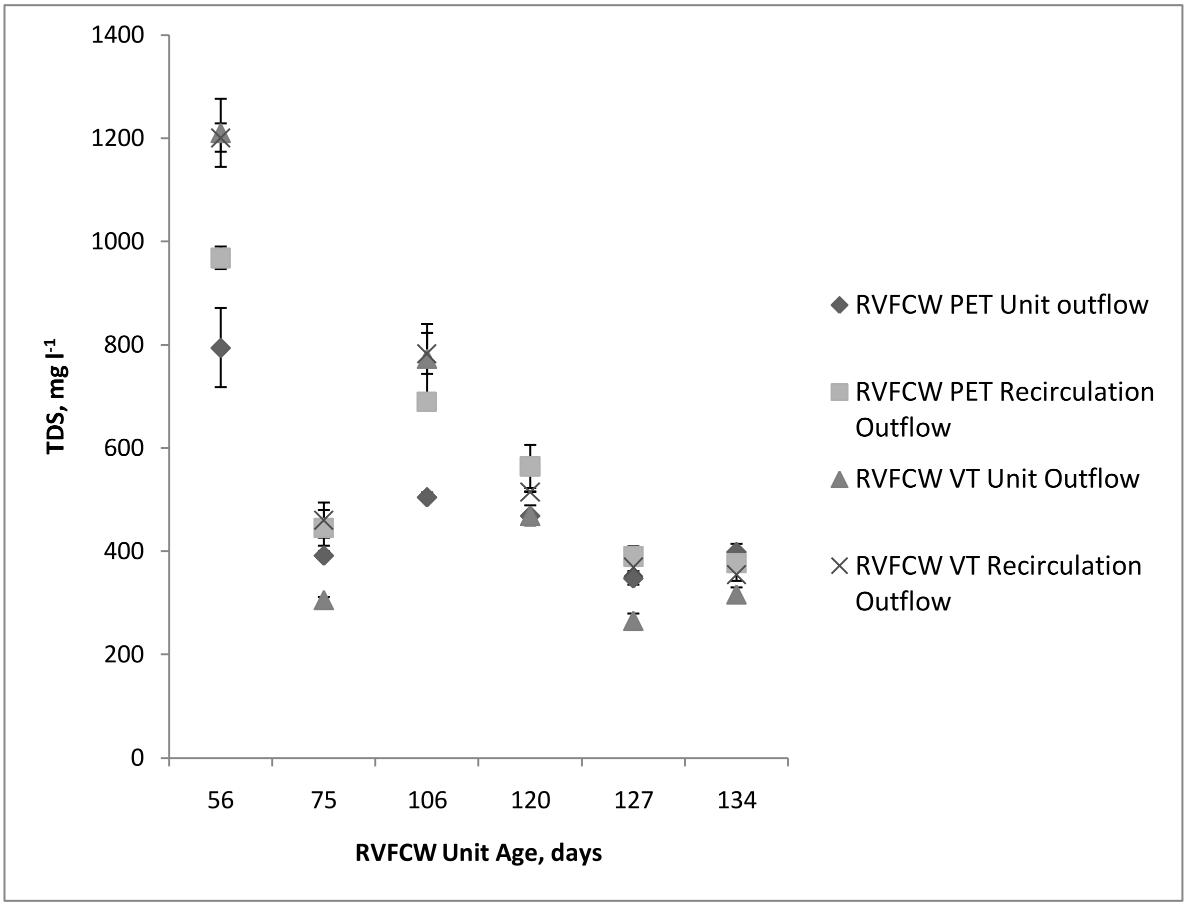

Figure 3.5: Recycled vertical flow constructed wetland (RVFCW) polyethylene terephthalate (PET) plastic and volcanic tuff (VT) unit total dissolved solids concentrations, with standard errors, over time measured in RVFCW unit outflow and recirculation outflow, during greywater treatment.

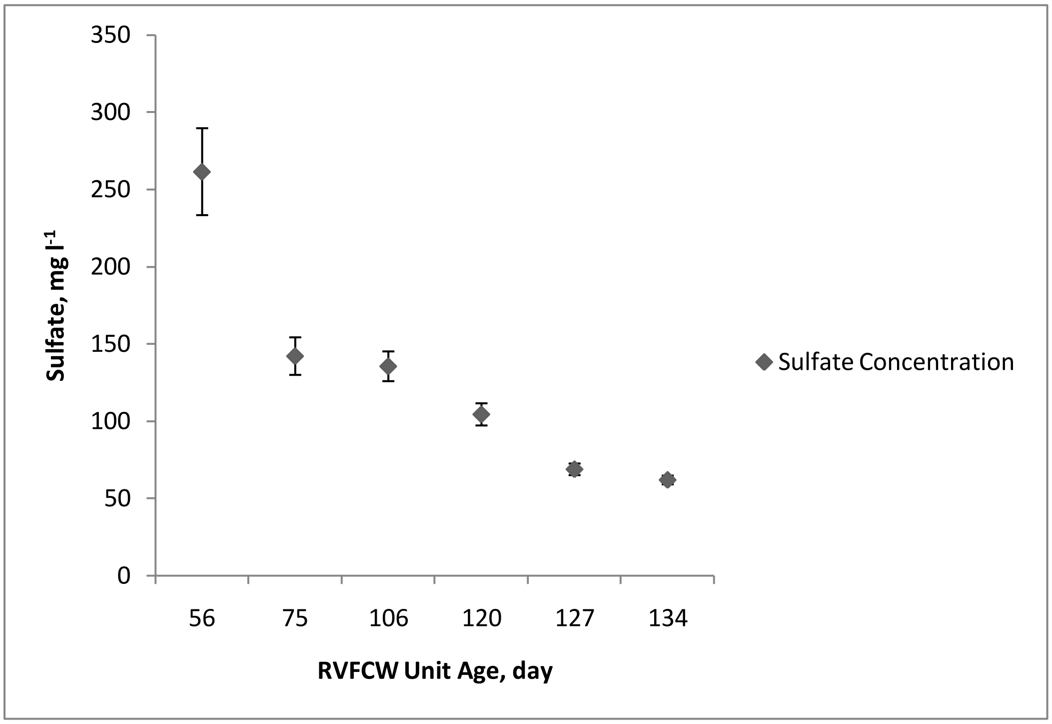

Figure 3.6: Recycled vertical flow constructed wetland (RVFCW) unit average sulfate values, with standard error) measured in RVFCW unit outflow and recirculation outflow, during the greywater treatment.

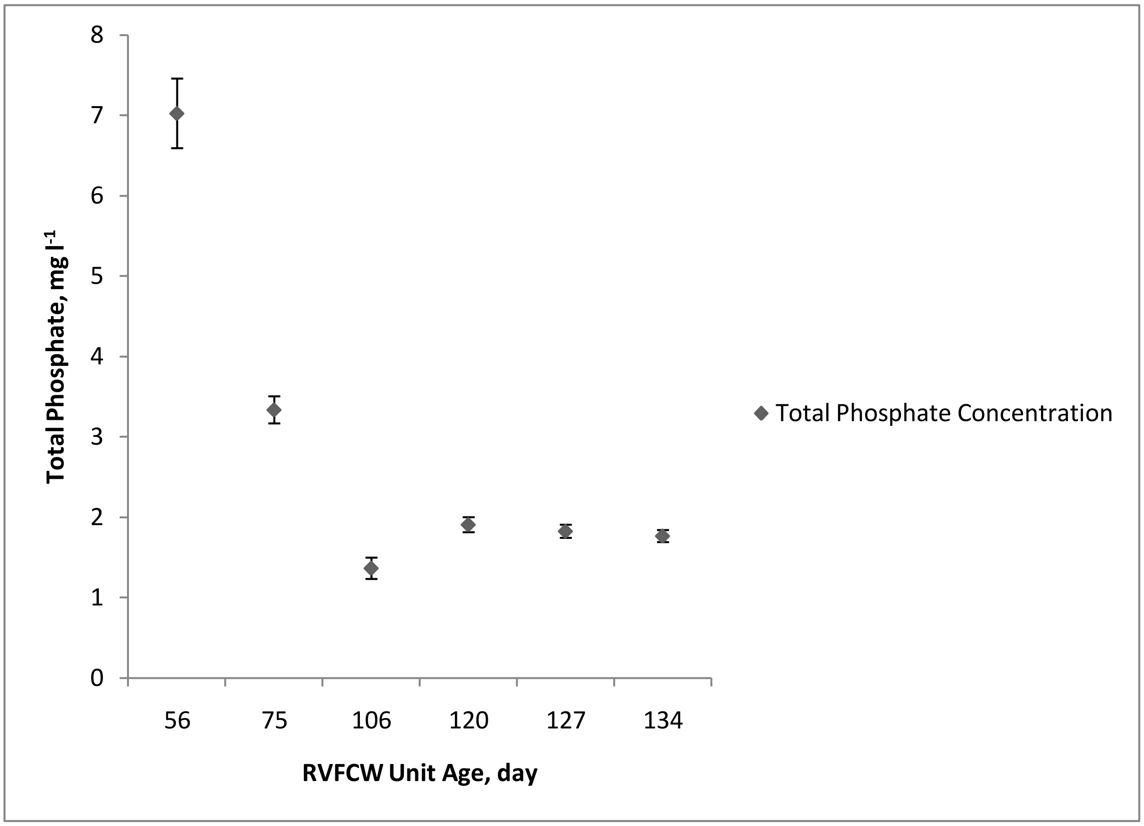

Figure 3.7: Total phosphate values averaged from all four sampling points (source, sedimentation outflow, RVFCW unit outflow, and recirculation outflow) over time, with standard errors, during the greywater treatment.

Chapter 4

Evaluation of a Portable Recycled Vertical Flow Constructed Wetland as a Treatment System for Dairy Wastewater

4.1 Abstract

The purpose of this study was to develop and evaluate a portable, recycled vertical flow constructed wetland (RVFCW) with a low surface area requirement, and low capital construction costs, which would achieve biologically acceptable contaminant removal efficiencies during treatment of high strength dairy wastewater (DWW). In addition, the research objective was to evaluate the treatment efficacy of RVFCW units constructed with recyclable PET plastic, to determine if the PET plastic could viably replace traditional gravel as the primary bed media. Eleven water quality monitoring parameters were chosen to determine RVFCW contaminant and nutrient removal capabilities: total phosphate (TP), ammonia-nitrogen, nitrate-nitrogen, total nitrogen (TN), total suspended solids (TSS), total dissolved solids (TDS), total organic carbon (TOC), biochemical oxygen demand (BOD), total plate count (TPC), fecal coliforms (FC), and *E. coli*. The RVFCW units showed a 17.2±30% decrease ($p < 0.0001$) in TP concentrations, a 70±11.7% ($p < 0.0001$) decrease in ammonia-nitrogen, and a 2028±1476% ($p < 0.0001$) increase in nitrate-nitrogen concentration. Total nitrogen concentrations decreased by 45.8±7.8% ($p < 0.0001$). Total suspended solids concentration decreased by 81±7.4% ($p < 0.0001$), and TDS concentrations increased by

26±41.2% ($p = 0.0319$). The units achieved a 68.1±8.8% decrease ($p < 0.0001$) in TOC concentrations during DWW treatment, and BOD decreased by 51±19.1% ($p < 0.0001$) during treatment. pH values increased by 9±2.1% ($p < 0.0001$) during GW treatment. No reduction in TPC counts were observed during dairy wastewater treatment, however a 0.4 log reduction in fecal coliforms was observed during treatment ($p < 0.0001$). The RVFCW units achieved a 1 log reduction in *E.coli* ($p < 0.0001$).

The two types of RVFCW units performed differently for the following parameters, including ammonia, nitrate and pH. The RVFCW units constructed with PET achieved a 65±11.7% reduction in ammonia-nitrogen concentrations ($p < 0.0001$), which was 15.4% ($p = 0.0398$) less than the ammonia-nitrogen reduction observed in the RVFCW VT units during DWW treatment. Conversely, the RVFCW PET units showed an overall increase in nitrate-nitrogen concentrations ($p < 0.0001$), which was 80% ($p < 0.0001$) less than the increase observed in the RVFCW VT units. RVFCW PET unit pH values showed a 30.7% smaller increase ($p = 0.0099$) than the RVFCW VT unit pH values, during DWW treatment.

The results of this study indicate that the RVFCW units would perform well as a primary or secondary treatment system for high strength agricultural wastewaters, if nutrients and solids reduction is a priority. The vertical flow design was not suitable for nitrate removal, but performed well in reducing ammonia concentrations.

Overall the systems performed well, indicating that the RVFCW units would be a viable, low-cost, and effective wastewater treatment system to improve the quality of agricultural wastewater before discharge into the environment. In addition, the reductions in total nitrogen, total phosphate, fecal coliforms and *E. coli* would indicate that when paired with an anaerobic treatment system such as horizontal flow constructed wetlands, the effluent from the RVFCW units would be suitable component for wastewater recycling and reuse.

The results of this study also indicate that PET can effectively replace gravel as a primary bed media without impairing treatment levels, thereby drastically reducing the capital cost of artificial wetland implementation.

4.2. Introduction

Eutrophication of fresh and salt surface waters in the U.S., as a result of agricultural runoff, costs \$2.2 billion a year (Dodds et al., 2009, Mitsch et al., 2001, Moore et al., 2010). The primary cause of eutrophication is an excess of nitrogen and phosphorus in agricultural runoff (Novotny, 2003). Hunt et al. (2004) reported that groundwater samples from a field sprayed with heavily loaded swine wastewater contained 77.99 mg N kg^{-1} soil, and that a stream lying contiguous with the spray field had a concentration of 32.01 mg N kg^{-1} soil. Sharpley et al. (1992) conducted a 5-yr study on the concentration of phosphorus in agricultural runoff from 20 agricultural plots throughout Oklahoma. One 2.7 ha plot, under conventional till wheat management, was reported to have an annual soil loss of 39 597 kg ha yr^{-1}, and a total phosphorus level of 14 899 kg ha yr^{-1} in the associated runoff. Mitigation of runoff from agricultural land is largely left to the producer, and is not regulated by the EPA. Wastewater from animal production facilities is typically stored and then sprayed onto fields in the dry season (Wood et al., 2007). This practice occurs at the detriment of soil and water quality (Brewer et al., 1999). Dairy wastewater is a particularly high-strength wastewater containing manure, urine, milk, cleaning products and silage effluent, with BOD_5

(biochemical oxygen demand measured after a 5 d incubation period) concentrations as high as 2811 mg l^{-1} (Wood et al., 2007). The land spreading of dairy wastewater, or the discharge of dairy wastewater into local surface waters, without pre-treatment, could put human and environmental health at risk.

Constructed wetlands have been used for nearly a century as a primary treatment system for agricultural wastewater (Kadlec and Wallace, 2010). Rousseau et al. (2004) collected data on over 107 constructed wetlands in use, in Flanders, finding that one of the primary uses for CWs was to treat dairy wastewater.

However, with many constructed wetland designs, treatment efficiencies for agricultural wastewater will often be limited due to poor wastewater distribution across the surface of the wetland, and low oxygen transfer rates (Sun et al., 2003). Vertical flow constructed wetlands are efficient at treating high strength wastewaters, such as dairy wastewater, because of the high oxygen transfer rate that occurs in the wetland media (Kadlec and Scott, 2009). Dairy wastewater is characterized by high concentrations of organic matter (Brewer et al., 1990, Van Kessel et al., 2002, Wood et al., 2007). Increased oxygen transfer, through the VFCW bed matrices, facilitates higher rates of organic matter degradation and microbial facilitated carbon compound transformations (Kadlec and Scott, 2009). In addition, nitrification of ammonia (found at high concentrations in dairy wastewater) occurs under aerobic conditions (Novotny, 2003, Kadlec and Scott, 2009, Van Kessel et al., 2002). Vertical flow constructed wetlands operating under fill and drain hydraulic modes can pull oxygen in from the atmosphere to replenish the biofilms with oxygen, aiding in nitrification (Sun et al., 2006). These attributes of VFCWs make them the ideal choice for treating dairy wastewater (Cooper et al., 1997, Kadlec and Scott, 2009, Wood et al., 2007)

4.3. Materials and Methods

4.3.1. Construction

A complete description of the construction of the RVFCW units, RVFCW bed matrix, RVFCW macrophytes and the grow lights is described in section 3.3.1. The descriptions of the sedimentation basins and the flow distribution pipes are provided in sections 3.3.2. and 3.3.3., respectively.

4.3.2. Recirculation Pump

Recirculation flow was provided to the subsurface irrigation system by a Pondmaster® (Danner Mfg, Inc., Islandia, NY) 190 gph submersible magnetic drive pump.

4.3.4. Wastewater Source, Transport, and Storage

The dairy wastewater used in this research was obtained from a 1500 hd dairy farm in northern Colorado. Water used to flush out the barns and the milking parlour flowed through two settling basins, into a weir which flowed into a lagoon. Water was collected at the end of the weir with a Flotec® (Delavan, WI) 4/10 HP sewage pump. The wastewater was pumped from the weir into a 450 gal transportation tank and taken to the RVFCW location, where the wastewater was pumped into a 1500 gal on-site storage tank.

4.3.5. Dilution

The dairy used for this research was experiencing difficulties with the settling basins used for dairy barn effluent sedimentation, at the time of the study. As a result, the DWW sampled at the end of the weir had a TSS concentration of 150 000 mg l^{-1}. This concentration of TSS in the DWW was considered too high to be applied directly to the constructed wetlands as VFCWs are prone to clogging, which greatly impairs wetland function (Hua et al., 2010, Kadlec and Scott, 2009, Langergraber et al., 2002, Sun et al., 2006). To choose an appropriate dilution factor, a small database of typical TSS concentrations from settled animal production wastewaters was compiled to determine a target mean concentration of TSS.

Healy et al. (2007) reported a TSS range of 133 – 1402 mg l^{-1}, while Wood et al. (2007) observed a TSS concentration of 6144 mg l^{-1}, for pre-settled dairy wastewater. Sun et al (2006) and Zhao et al. (2004) reported TSS levels for pre-settled swine production wastewater at 5540 mg TSS l^{-1} and 644 mg TSS l^{-1}. Based on this information, the DWW was diluted (with clean tap water) to achieve final TSS concentrations of 5 000 mg TSS l^{-1}. Sixty gal of dairy wastewater was poured into the 1500 gal storage tank, which was then filled with tap water to the 1500 gal mark.

4.3.6. Study Design

Dosing Patterns

Prior to filling the sedimentation basins, the water in the 1500 gal storage tank was mixed for a minimum of 10 min with the sewage pump. A minimum of 24 hr prior to dosing the RVFCW units with 350 l of DWW, the sedimentation basins were filled with diluted dairy wastewater. On a dose day, 350 l of DWW was released from the sedimentation basins onto each of the RVFCW units attached to the tank. During the second phase of the study, the ball valves were opened 15° off closed position, and 350 l was fed to an RVFCW unit. The ball valve connecting the sedimentation basin to the RVFCW unit was left open until approximately 350 l of DWW had flowed into the RVFCW. After the initial dose of 350 l was completed, the 190 gph submersible pond pumps were turned on for 16 hr to recirculate the water from the unit reservoir back to the submersed irrigation system in the RVFCW unit. Dosing took place on six dates from October to November, 2010 when the RVFCW units were the following ages: 238 d, 250 d, 257 d, 267 d, 275 d, 280 d.

Hydraulics

The average hydraulic retention time (HRT) and hydraulic loading rate (HLR) for all four RVFCW units was 0.059±0.0155 d^{-1} and 0.77±0.229 m d^{-1}. The average recirc-ulation time was 6.87±0.503 m^3 d^{-1}. Calculating HLR and HRT for the second phase of the study was based on a time average flow rate as described by Kadlec and Scott (2009), and is appropriate for HRT and HLR approximations for intermittent feed VFCWs. Hydraulic retention time and hydraulic loading rate were calculated by the method described in the following paragraph.

The ball valves connecting the sedimentation basins to the RVFCW unit were opened until the approximate depth of effluent measured in the RVFCW reservoirs had reached 0.27 m, and then were closed assuming that the water remaining in the wetland matrix would add an additional 0.025 m of depth, which would equal a total volume in the reservoir approximately equal to 350 l. The flow was timed from the time the ball valve was opened to the time surface ponding disappeared from the RVFCW unit. The time averaged flow rate was calculated with the following equation:

$$Q = \frac{V}{T} \tag{4.1}$$

Where Q is time averaged flow rate ($m^3\ d^{-1}$), V is the total volume dosed (m^3), and T is the total time measured for the dose (d). Hydraulic loading rate was then calculated with equation 3.2, and HRT was calculated with equation 3.3

Sampling Method

Water sampling was conducted at four different locations within the wastewater treatment flow stream: source (northern Colorado dairy farm), sedimentation basin outflow, RVFCW unit outflow, and recirculation outflow. At each sampling time, eight bottles of sample water was taken for analyses of fecal coliforms (FC), total plate count (TPC), *E. coli*, nitrate-nitrogen (NO_3^- – N), ammonia-nitrogen (NH_3 - N), total phosphate (TP), total organic carbon (TOC), total nitrogen (TN), biochemical oxygen demand (BOD), total suspended solids (TSS), total dissolved solids (TDS), and pH. Separate acid washed bottles were used for the collection of sample water used for the analyses of TP, NO_3^- – N, and NH_3 - N. At the dairy, the dairy wastewater was diluted with de-ionized water directly in the sampling bottle until the diluted DWW sample reached a concentration of 5 000 mg TSS l^{-1}. The water samples used for the analyses of TP, NO_3^- – N, and NH_3 - N were acidified to a pH less than 2, with 1M hydrosulfuric acid (H_2SO_4), for preservation. Each bottle was placed into a cooler with ice packs for transportation. The water samples were stored at 4°C until analyzed.

4.3.7. Sample Analyses

A detailed description of the colorimetric procedures used for the analyses of total phosphate, nitrate-nitrogen, ammonia-nitrogen, and total iron is provided in section 3.3.6.

Gravimetric Procedures

Total Suspended Solids

Total suspended solids (TSS) were determined using an adaptation of Method 2540 from the 21st edition of Standard Methods for Examination of Water and Wastewater (Eaton et al., 2005). The water samples were stored at 4°C until date of analyses, and then warmed to room temperature. For the dairy wastewater, a pre-filtration step was added due to the high concentration of large diameter solids. A 4 in × 4 in square of class 1000, optical grade cheesecloth (Thermo Fisher Scientific, Rochester, NY) was desiccated in a convection oven at 110°C for 1 hr. At the end of 1 hr, the cheesecloth was removed and cooled in a desiccator to room temperature. At the same time, a Millipore® (Billerica, MA) 47 mm diameter, binder-free, glass fiber filter, with 1µm porosity, was desiccated in a convection oven at 110°C for 1 hr, and cooled in a desiccator until room temperature. The filter and cheesecloth were weighed, and the cheesecloth placed over the top of a 250 ml glass graduated cylinder. The sample bottle was inverted 10 times and then 15 ml of DWW sample was poured through the cheesecloth into the graduated cylinder. The cheesecloth was gently squeezed of excess water over the graduated cylinder, placed in a 56 ml aluminum weigh dish, and then dried in a convection oven for 12 hr at 110°C. The Millipore® filter was placed into 250 ml capacity, Nalgene® (Thermo Fisher Scientific) polysulfone filter cartridge holder with receiver. The 15 ml of pre-filtered DWW was poured over the top of the filter, and the filter cartridge was connected to an air flow manifold. A vacuum pump was turned on to create suction, and the 15 ml of DWW was pulled through the filter. The filter was removed from the filter holder after the 15 ml had passed through to the receiver, and placed in a pre-dried, pre-weighed aluminum weigh dish. The filter was dried in a convection oven at 110°C for 12 hr. At the end of the drying period, the filter and the cheesecloth was removed and placed in a desiccator to cool to room temperature, and weighed again. The concentration of TSS was calculated with the following equation:

$$\text{mg l}^{-1} \text{ of total suspended solids} = \frac{((A-B)+(C-D))x\ 1000}{Sample\ Volume, ml} \qquad (4.4)$$

Where A is the weight of the filter + dried residue (mg), B is the weight of the filter (mg), C is the weight of the cheesecloth and dried residue (mg), and D is the weight of the cheesecloth (mg).

Samples from the sedimentation basin outflow, RVFCW unit outflow, and recirculation outflow were analyzed using the method described in section 3.3.6 for TSS, except that the sample volume used for analysis was 25 ml and not 250 ml, due to the high solids content of the sample water.

Total Dissolved Solids

Total dissolved solids (TDS) were determined using an adaptation of Method 2540 from the 21st edition of Standard Methods for Examination of Water and Wastewater (Eaton et al., 2005). Total dissolved solids for DWW water was measured in conjunction with TSS, and followed the same protocol as described in the previous section on TSS analysis of DWW water. Total dissolved solids measurement for the DWW water, and all other sample water followed the same protocol as detailed in section 3.3.6.

High Temperature Combustion

Total Organic Carbon and Total Nitrogen

A description of the protocol used to analyze total organic carbon is provided in section 3.3.6.

Total nitrogen was analyzed using an adaptation of Method 4500-N from the 21st edition of Standard Methods for Examination of Water and Wastewater (Eaton et al., 2005). Samples were analyzed with a Shimadzu® TNM-1 nitrogen module attached to a TOC-VCSH instrument, utilizing high temperature combustion oxidation with chemiluminescence detection. Samples were stored at 4°C until time of analyses. The samples were warmed to room temperature, and inverted 10 times to mix, before approximately 30 ml of water sample was poured into a Shimadzu® ASI-V auto-sampler sample cell. The instrument auto-calibrated with internal software based on two nitrogen standard concentrations: 1 mg l^{-1} and 10 mg l^{-1}.

Microbial Analyses, Statistical Analysis, and pH

Total plate count and fecal coliforms were measured as described in section 3.3.6. *E. coli* counts were obtained simultaneously with fecal coliforms. All blue colored colonies found on the Fecal Coliform Petrifilm™ (3M©) were identified as *E. coli*.

Description of the procedures used for the statistical methods of analysis can also be found in this section 3.3.6. The procedure for pH measurement can be found in this section as well.

4.4 Results

4.4.1. Ammonia-Nitrogen

The RVFCW units achieved excellent removal efficiencies of ammonia-nitrogen, with overall concentrations decreasing by 70±11.7% ($p < 0.0001$) during treatment (See Table 4.1). The DWW ammonia- nitrogen concentration was 67% higher ($p < 0.0001$) on the first day of sampling than the average DWW concentrations measured on all subsequent sampling days. The elevated ammonia-nitrogen concentration had no statistically significant impact on RVFCW unit treatment efficiency. RVFCW PET Unit 1 showed significantly higher ammonia-nitrogen concentrations in the RVFCW unit outflow and recirculation outflow than the other RVFCW units, achieving only a 56.6±4.8% ammonia-nitrogen reduction ($p < 0.0353$). Ammonia-nitrogen concentration was one of the few water quality parameters affected by the difference in RVFCW unit media (See Figure 4.1). The RVFCW PET units removed a smaller percentage (65±11.7%, $p = 0.0411$) of ammonia-nitrogen than did the RVFCW VT units (75±9.8%, $p = 0.0411$) (See Table 4.2).

4.4.2. Nitrate-Nitrogen

The RVFCW units showed an increase in nitrate-nitrogen levels through the treatment process (2028±1476%, $p < 0.0001$). RVFCW PET Unit 1 had the smallest increase in nitrate-nitrogen concentrations during DWW treatment (940±624%, $p < 0.0001$). Nitrate-nitrogen concentration was the second parameter impacted by the bed media type (See Figure 4.1). Nitrate-nitrogen concentrations were not as high ($p < 0.0001$) in the RVFCW PET units at 3.09±1.7 mg l^{-1} ($p < 0.0001$), as they were in the

RVFCW VT units (5.37±1.68 mg l^{-1}, $p < 0.0001$) (See Table 4.2). Nitrate effluent concentrations increased after recirculation for all RVFCW units (See Table 4.1).

4.4.3. Total Nitrogen

A significant decrease in TN values was observed throughout the DWW treatment. The total TN reduction was measured at 45.8±7.8% ($p < 0.0001$), with sedimentation contributing to 28±5.8% reduction ($p < 0.0001$) of the initial TN reduction. The RVFCW units achieved an additional 28±5.8% reduction, with recirculation lowering the TN concentration to a final value of 55.53±7.84 mg l^{-1} ($p < 0.0001$) (See Table 4.1). No secondary treatment effect on TN removal efficiencies was observed.

4.4.4. Total Suspended Solids

The RVFCW units showed an 81±7.4% reduction ($p < 0.0001$) in TSS concentrations during DWW treatment. There was a steady decrease through each step of treatment from sedimentation through recirculation. Sedimentation accounted for an initial 67±9.7% reduction in TSS concentration ($p < 0.0001$). The RVFCW units accounted for an additional 9.2±27% reduction of the sedimentation TSS outflow values ($p < 0.0001$). Final TSS concentrations were 122.7±48.27 mg l^{-1} after recirculation ($p < 0.0001$) (See Table 4.1). No secondary treatment effect was observed .

4.4.5. Total Dissolved Solids

An overall increase in TDS by 26±41.2% ($p < 0.0319$) was observed during DWW treatment, with no secondary treatment effect demonstrated (See Table 4.1). There was a statistically significant spike in TDS DWW concentrations on the second day of sampling. The DWW TDS concentrations were 24% higher ($p < 0.0001$) at the second sampling event than on the first and third on-site sampling event (See Figure 4.2). The elevated DWW TDS concentrations correlated with higher effluent concentrations from the sedimentation basins and the RVFCW units. There was no secondary treatment effect observed.

4.4.6. Total Phosphate

Total phosphate effluent levels decreased by 17.2±30% ($p < 0.0001$), with sedimentation acting as the predominant removal mechanism during treatment (See Table 4.1). The RVFCW units demonstrated a small amount TP removal (0.7±13.9% of sedimentation outflow concentrations), however the process of recirculating the RVFCW outflow increased TP RVFCW outflow concentrations by 8.4±20% (See Table 4.1).

4.4.7. Total Plate Count, Fecal Coliforms and E.Coli

The RVFCW units demonstrated no reduction in total plate counts (TPC) during the duration of the trial. TPC remained at 10^6 CFU ml^{-1}, during treatment. FC counts showed a statistically significant 0.4 log reduction ($p < 0.0001$). The RVFCWs achieved a 1 log reduction of *E. coli*, however the largest and most significant reduction occurred during sedimentation (See Table 4.1). There was no secondary treatment effect demonstrated for FC or *E.coli*.

4.4.8. Total Organic Carbon

Total organic carbon concentrations decreased by 68.1±8.8% ($p < 0.0001$), throughout DWW treatment. Sedimentation resulted in an initial 49±11.2% decrease ($p < 0.0001$) in TOC concentration (See Table 4.1). No secondary treatment effect was demonstrated.

4.4.9. Biochemical Oxygen Demand

The DWW averaged 49±11.2 mg BOD 1^{-1} during the trial, and was reduced to a final BOD concentration of 92.21±35.40 mg l^{-1} in the recirculation effluent, for a total reduction of 51±19.1% ($p < 0.0001$) during treatment (See Table 4.1). No secondary treatment effect was demonstrated.

4.4.10. pH

pH levels of the diluted DWW averaged 7.2±0.14 and rose at each level of treatment, to a final pH value of 8.29±0.20 (See Table 4.1). There was no significant change at any point in treatment, at any sampling point, or for any RVFCW unit during the trial. A secondary treatment effect was observed, with the RVFCW PET units demonstrated a 30.7% less increase ($p = 0.0090$) in pH levels than the RVFCW VT units (See Table 4.2).

4.4.11. Total Iron

The total iron concentrations remained at 0.15 mg l^{-1} in the RVFCW and recirculation effluent, for the duration of the trial. At this level, total iron concentrations did not interfere with the colorimetric procedures.

4.4.12. Hydraulics

No correlation was found between outflow concentrations of any of the parameters analyzed with either HRT or HLR, or recirculation rate. There was no statistically significant difference between the two RVFCW unit types and HRT, HLR or recirculation rate.

4.5. Discussion

Constructed wetlands have been used to treat agricultural wastewater for many decades throughout Europe, Great Britain and the United States. CWs have been used to treat sugar beet processing wastewater, dairy parlour wastewater, pig production slurry, and fertilizer plant run-off (Cooper et al., 2010, Morris and Herbert, 1997, Wood et al., 2007, Zhao et al., 2004). Vertical flow constructed wetlands are especially attractive as an agricultural wastewater treatment system. Vertical flow constructed wetlands can achieve high oxygen transfer rates, which aids in the reduction of biochemical oxygen demand, ammonia, and suspended solids (Kadlec and Scott, 2009, Rousseau et al., 2004). In addition, the complex biological processes occurring in the artificial wetland sediments require abundant external sources of nitrogen and carbon, and therefore aid in the reduction of nutrient discharge into surface waters (Kadlec and Scott, 2009).

Because the face of modern agriculture has changed dramatically over the last century, moving from small subsistence farming and ranching, to large scale, corporate owned farms and concentrated animal feeding operations (CAFOs), concern has arisen over how to secure food for the future sustainably. Constructed wetlands can be a critical component in this challenge.

In 1988, the American Society of Agronomy stated that sustainable agriculture must be economically viable as well as enhance environmental quality (White et al., 1994). Pesticides, herbicides, fertilizers, and manure greatly alter the environmental quality of the local soils and surface waters (Cooper et al., 2010, Hunt et al., 2004, Muller et al., 2000). The discharge of these pollutants comes at great economic cost, which is largely externalized due to the nature of watershed interconnectivity (Novotny, 2003).

The nature of agricultural practices creates a hugely expansive decentralized community of croplands, rangelands, and CAFOs. Any treatment system for agricultural wastewater must be on-site, low-cost, and low maintenance. Vertical flow constructed wetlands are particularly attractive as an on-site treatment system, as these CWs require the least amount of area for land conversion (Rousseau et al., 2004). The small land requirement for implementation means that valuable cropland can remain intact, a run-off treatment system can be installed at multiple locations within a feedlot to mitigate rainfall driven events, and CAFOs in which animals are raised indoors, such as in swine and poultry operations, can construct VFCW systems inside for year-round wastewater treatment.

The objective of this research was to develop an agricultural wastewater treatment technology which could treat high strength wastewaters to biologically acceptable discharge levels, or to a level appropriate for reuse. The VFCW developed for this research was constructed to maximize oxygen transfer, as well as reduce the probability of clogging due to the elevated levels of TSS found in dairy wastewater (Hua et al., 2010, Wood et al., 2007).

The secondary objective of this research was to find cheaper construction materials to replace traditional gravels and sands, in effort to reduce the initial capital cost of the artificial wetlands without compromising effluent water quality. Polyethylene terephthalate (PET) plastic was chosen as the alternative substrate for the RVFCW units. Eleven wastewater quality parameters were chosen for analysis to represent the treatment efficacy of the RVFCW units used in this research. The analysis of the data obtained during the trial period would aid in the determination of the RVFCW units' potential as an effective agricultural wastewater treatment system, and if recycled PET plastic could effectively replace volcanic tuff.

4.5.1. Total Nitrogen, Ammonia-Nitrogen, and Nitrate-Nitrogen

Total nitrogen includes nitrate, ammonia, oxidized nitrogen, and organic nitrogen. Nitrogen undergoes extensive transformation by a variety of mechanisms, such as ammoniafication (mineralization), nitrification, denitrification, assimilation, and decomposition (Kadlec and Scott, 2009). Plant and microorganism growth will account for the reduction of nitrogen in wetland environment by assimilation (Kadlec and Scott, 2009). Another mechanism for nitrogen reduction is the volatilization of ammonia. Un-ionized ammonia (NH_3) is highly volatile, and will move through the wetland matrix by diffusion, and then mass transfer at the water surface into the atmosphere (Kadlec and Scott, 2009). Although the RVFCW units designed for this research will reduce total nitrogen concentrations through predominately aerobic processes, or by volatilization, there is a possibility that anaerobic conditions in the topsoil layer were achieved during the 16 hr recirculation period. If anaerobic conditions were achieved, existing research states that sulfur-driven autotrophic denitrifciation or carbon-driven heterotrophic denitrification can reduce nitrate to nitrogen gas (Kadlec and Scott, 2009). Nitrogen gas is readily transferred into the atmosphere (Novotny, 2003).

Although the RVFCW PET unit outflow nitrate-nitrogen concentrations were lower than the RVFCW VT unit outflow nitrate-nitrogen, RVFCW PET Unit 1 demonstrated significantly lower outflow nitrate-nitrogen concentrations, at 0.98±0.36 mg l^{-1}, than RVFCW VT Unit 4 (See Figure 4.1). This result indicates that the conditions in the RVFCW PET unit 1 bed matrix were more hypoxic than in the RVFCW PET unit

4. The anaerobic conditions in RVFCW PET unit 1 may be attributable to the high quantity of soil loss from the topsoil layer, which was the highest loss of all four units. As the average TSS and TDS content was no higher in the RVFCW Unit 1 outflow than the other units, and because excessive surface ponding with subsequent RVFCW unit overflow into the reservoir can account for elevated TSS concentrations measured after the recirculation period, the majority of the eroded soil must have become entrapped in the middle layer. Essentially, this action filled the space between PET plastic sections with topsoil, creating pore space for greater water retention, as the capillary pressure in the topsoil is greater than the capillary pressure in the PET matrix. The retention of water in the pore space will facilitate anoxic conditions, due to the lower oxygen transfer rate and pore water oxygen consumption by microorganisms. This mechanism may have allowed for the process of denitrification to occur at a greater rate than in the other three units. However, the difference in treatment removal efficiencies between the RVFCW types is less pronounced after recirculation has occurred, supporting the hypothesis that the mechanism of recirculation re-aerates the wetland bed matrix (See Figure 4.1).

The overall performance of the RVFCW units for reductions in ammonia concentrations supports existing literature on ammonia fate processes in vertical flow constructed wetlands. The RVFCW units demonstrated higher removal efficiencies for the constructed wetlands presented by Morris and Herbert (1997) and Sun et al. (2006) used to treat sugar beet processing wastewater and pig slurry, respectively. Both types of wastewater was characterized by high concentrations of ammonia (201 mg NH_4^+-N l^{-1} for pig slurry, and 46 mg NH_3-N l^{-1} in sugar beet processing wastewater), and were treated with vertical flow constructed wetlands. Morris and Herbert (1997) reported a

63% reduction in ammonia-nitrogen concentrations, and Sun et al. (2006) reported a 4.47% ammonia reduction. These results compare to the 70±11.7% reduction in ammonia concentrations observed with the RVFCW units used in this research, during dairy wastewater treatment, with influent ammonia-nitrogen concentrations of 47.55±11.16 mg l^{-1}.

4.5.2. Solids and Total Phosphate

Discharge of phosphate from dairy barns and parlours are directly related to the feedstuffs provided to lactating cows and growing heifers, and therefore directly related to the solids concentration in dairy wastewater. According to Harris et al. (1990), lactating cattle need approximately 25.4 g d^{-1} of phosphorus, a mineral they are fed in the form of meals, such as cottonseed meal, distillers grain, rice bran and soybean meal. At the height of lactation, dairy cows may excrete phosphorus levels as high as 112 g d^{-1} through feces, milk and urine. The DWW trial demonstrated a decrease in TP concentrations during treatment, as opposed to the GW trial TP concentrations which increased during treatment. The conflicted results may indicate that the hydraulic program employed during the greywater trial facilitated elevated TP background levels. In addition, the TSS concentrations were reduced by 81±7.4%, which supports the evidence that TP removal from DWW will occur through interception of suspended particles to which the phosphorus is adsorbed.

The performance of the RVFCW units, in terms of total phosphorus reduction, is well within the range reported for vertical flow constructed wetlands constructed wetlands (Kadlec and Scott, 2009). Knight et al. (2000) reported that average total phosphorus treatment efficiencies were approximately 48% for 135 constructed wetlands surveyed in North America, treating livestock wastewater. The RVFCW units achieved only 23.8±23.8% TP reduction from the source TP dairy wastewater concentrations to RVFCW outflow, indicating the need for improving phosphorus treatment efficiencies of these RVFCW units.

4.5.3. Total Plate Count, Fecal Coliforms, and E. coli

Total plate count, fecal coliform and *E. coli* counts were much lower than expected for dairy wastewater. Kadlec and Scott (2009) stated that cows can produce 10^5 – 10^7 fecal coliforms per gram of fecal material. Fecal coliform and *E. coli* counts were no higher than 10^3 – 10^4 CFU ml^{-1} during the DWW trial. The unexpectedly low TPC, FC and *E. coli* counts may be attributable to the use of Rumensin® (Elanco, Greenfield, IN) at the dairy used for the trial. Rumensin® is an ionophore that can be classified as an antibiotic, and is fed to cattle in the form of sodium salts (CSU, 2011). In addition, water samples were taken from the dairy during the months of October and November. The sampling location was downstream from a settling basin, which would leave the water exposed to cold temperatures, and therefore limit fecal coliform growth.

Total plate count influent concentrations were most likely as low as the natural background levels of the RVFCWs units, as the final average TPC levels measured in the recirculation outflow for the GW and DWW trials were the same, 10^5 CFU ml^{-1}.

4.5.4. Total Organic Carbon and Biochemical Oxygen Demand

Both biochemical oxygen demand (BOD) and TOC are used as indicators of water quality (Kadlec and Scott, 2009). Biochemical oxygen demand is the measure of oxygen consumption of microorganisms during the oxidation of organic matter, represented by total organic carbon concentrations (Kadlec and Scott, 2009). The organic matter present in dairy wastewater is predominately fecal material, silage, bedding, milk (Wood et al., 2007). These large organic particulates are rapidly used in the wetland environment, and broken down from starches, sugars and cellulose to amino acids and fatty acids (Kadlec and Scott, 2009). In FWS and HSSF wetlands, carbon processing will proceed from aerobic respiration to anaerobic processes such as fermentation, denitrification, iron reduction, sulfate reduction and ultimately methanogenesis. The RVFCW units developed for this research are characterized by a highly oxygenated environment, meaning that oxygen consumption by resident microorganisms in the wetland will create a BOD plateau effect, much like the one described by Nolte and associates (1997). This BOD plateau would be considered as a natural background level, since plant litter and microorganism death will continue to provide new sources of organic carbon, once external carbon sources have completely decomposed (Kadlec and Scott, 2009).

The BOD treatment efficiencies of the RVFCW units are well within the range, at 51±19.1%, presented in literature for vertical flow constructed wetlands constructed wetlands (Kadlec and Scott, 2009). Sun et al. (2006) reported a 9.8% BOD reduction with a single vertical flow constructed wetland cell receiving pig slurry effluent, with an 82% BOD reduction achieved only after the effluent had flowed through five constructed wetland cells. Wood et al. (2007) reported a75% BOD treatment efficiency for a subsurface flow constructed wetland receiving dairy wastewater, which had been pretreated with an intensive aeration unit.

4.6. Summary

The RVFCW units used in this study performed well for nine of the eleven parameters analyzed , including total organic carbon (TOC), biochemical oxygen demand (BOD), fecal coliforms (FC), *E. coli*, ammonia-nitrogen, total nitrogen (TN), total suspended solids (TSS), total phosphate (TP), and pH.

The increase in outflow nitrate-nitrogen concentrations, seen during the trial period, suggests that the RVFCW units should be used in series with another wastewater treatment method (HSSF wetlands) if influent wastewater is characterized by elevated nitrate-nitrogen, and if nitrate reduction is a primary treatment goal (Kadlec and Scott, 2010). Although the RVFCWs demonstrated exceptional removal efficiencies in the majority of the target parameters, the RVFCW effluent was still heavily contaminated with nitrogen and phosphorus, the primary pollutants of concern for agricultural wastewater discharge. Although the RVFCWs would provide a much better solution to untreated agricultural wastewater discharge than the lack of a treatment system all together, a true commitment to environmentally sustainable agriculture in the United States and Canada requires a higher effluent standard than can be provided by these RVFCW units as stand-alone treatment systems. A horizontal subsurface flow constructed wetland receiving effluent from a RVFCW unit, may aid in further reducting the nutrient concentrations in the RVFCW unit effluent (Noorvee et al., 2005). Research done by Weber et al. (2007) and Chazarenc et al. (2007) indicates that the incorporation of arc furnace steel slag into the artificial wetland bed matrix, or as a pre-filter, can increase phosphorus treatment efficiencies. Zhao et al. (2008) provided evidence that dewatered alum sludge cakes could be incorporated in the wetland bed media and facilitate the reduction of phosphorus effluent levels.

The results of this research indicate that a RVFCW unit can achieve appreciable levels of contaminant reduction, and can be a viable and important component in the treatment of agricultural wastewaters prior to discharge or reuse. The results of this research also indicate that PET plastic can successfully replace traditional gravels as primary artificial wetland bed material without compromising treatment efficacy. The decreased surface area need of these RVFCWs make them an attractive choice for indoor animal production facilities, multi-point sources at feedlots, and impoverished communities with high human and animal population densities utilizing the same surface water body for domestic, agricultural, and industrial needs.

Appendix C

Table 4.1: Average values for target parameters measured at the four sampling points during dairy wastewater treatment, reported with percent removals after each treatment stage and total percent removal for the RVFCW treatment system. A decrease in parameter concentrations during greywater treatment is indicated by a positive percent removal, and an increase in parameter concentrations is indicated by a negative percent removal.

Parameter	Dairy Wastewater*	Sedimentation Basin Outflow	% Removal	RVFCW† Unit Outflow	% Removal	Recirculation Outflow	% Removal	% Total Removal
Ammonia-N	47.55±11.16	37.43±6.59	-18±17.6	21.93±7.13	-41±18	13.65±5.43	-37±16.7	-70±11.7
Nitrate-N	0.23±0.06	0.27±0.22	+25±85	3.03±1.57	+1625±1400	4.23±2.02	+56±69.6	+2028±1476
Total Nitrogen	102.7±3.64	73.4±5.15	-28±5.8	64.15±6.18	-12±11.8	55.53±7.84	-13±11.4	-45.8±7.8
Total Phosphate	9.48±1.28	7.17±1.6	-23±20.8	7.03±1.49	-0.7±13.9	7.57±1.86	+8.4±20	-17.2±30
Total Organic Carbon	529.6±33.57	267.3±60.7	-49±11.2	217.6±38.9	- 16±16.6	168.5±46.33	-22±20.5	- 68.1±8.8
Biochemical Oxygen Demand	191.5±17.10	109.4±31.5	-42±16.4	97.66±21.2	-7±18.8	92.21±35.40	-3.5±39	-51±19.1
Total Suspended Solids	646.1±20.37	213.8±59.7	-67±9.7	185.5±45.8	-9.2±27	122.7±48.27	-33±19.5	-81±7.4
Total Dissolved Solids	747.2±80.10	832.5±228	+11.2±31	902.4±230	+19.6±58	928.6±279.6	+2.6±34.4	+ 26±41.2
Total Plate Count	$1.78\times10^6\pm6\times10^5$	$2.5\times10^6\pm2.6\times10^6$	-	$1.38\times10^6\pm8\times10^5$	-	$3.2\times10^5\pm4.6\times10^6$	-	-
Fecal Coliform	$4.9\times10^3\pm4\times10^2$	$1.3\times10^3\pm1.2\times10^3$	-0.6 log	$1.7\times10^3\pm1.1\times10^3$	+ 0.1 log	$1.8\times10^3\pm2.2\times10^3$	-	- 0.4 log
E. coli	$3.1\times10^3\pm8\times10^2$	$5.2\times10^2\pm6.5\times10^2$	-1 log	$3.59\times10^2\pm4\times10^2$	-	$4.52\times10^2\pm6\times10^2$	-	- 1 log
pH	7.6±0.14	8.13±0.13	+7±1.5	8.18±0.14	+ 0.67±1.3	8.29±0.20	+ 1.3±1.4	+ 9±2.1

* All values are reported as mg l accept for total plate count, fecal coliforms, and *E. coli* which are reported as CFU ml^{-1}, and pH

† Recycled vertical flow constructed wetland

Table 4.2: Comparison of ammonia, nitrate, and pH levels, measured during dairy wastewater treatment trial, between the recycled vertical flow constructed wetland (RVFCW) units constructed with polyethylene terephthalate plastic as the primary wetland bed media and the RVFCW units constructed with volcanic tuff as the primary bed media. A decrease in parameter concentrations during greywater treatment is indicated by a positive percent removal, and an increase in parameter concentrations is indicated by a negative percent removal.

Parameter	Dairy Wastewater*	Sedimentation Basin Outflow	RVFCW† PETǂ Unit Outflow	RVFCW VT§ Unit Outflow	RVFCW PET Recirculation Outflow	RVFCW VT Recirculation Outflow	RVFCW PET Unit Total Removal %	RVFCW VT Unit Total Removal %
Ammonia-Nitrogen	47.55±11.16	37.43±6.59	25.99±6.43	17.87±5.37	16.08±5.86	11.22±3.81	- 65±11.7	- 75±9.8
Nitrate-Nitrogen	0.23±0.06	0.27±0.22	2.10±1.45	3.96±1.09	3.09±1.7	5.37±1.68	+1449±1069	+2607±1605
pH	7.6±0.14	8.13±0.13	8.11±0.13	8.25±0.1	8.2±0.19	8.38±0.18	+7.9±1.8	+10.3±1.6

* All values reported in mg l^{-1}, except pH.
†Recycled vertical flow constructed wetland
ǂ Polyethylene terephthalate
§ Volcanic tuff

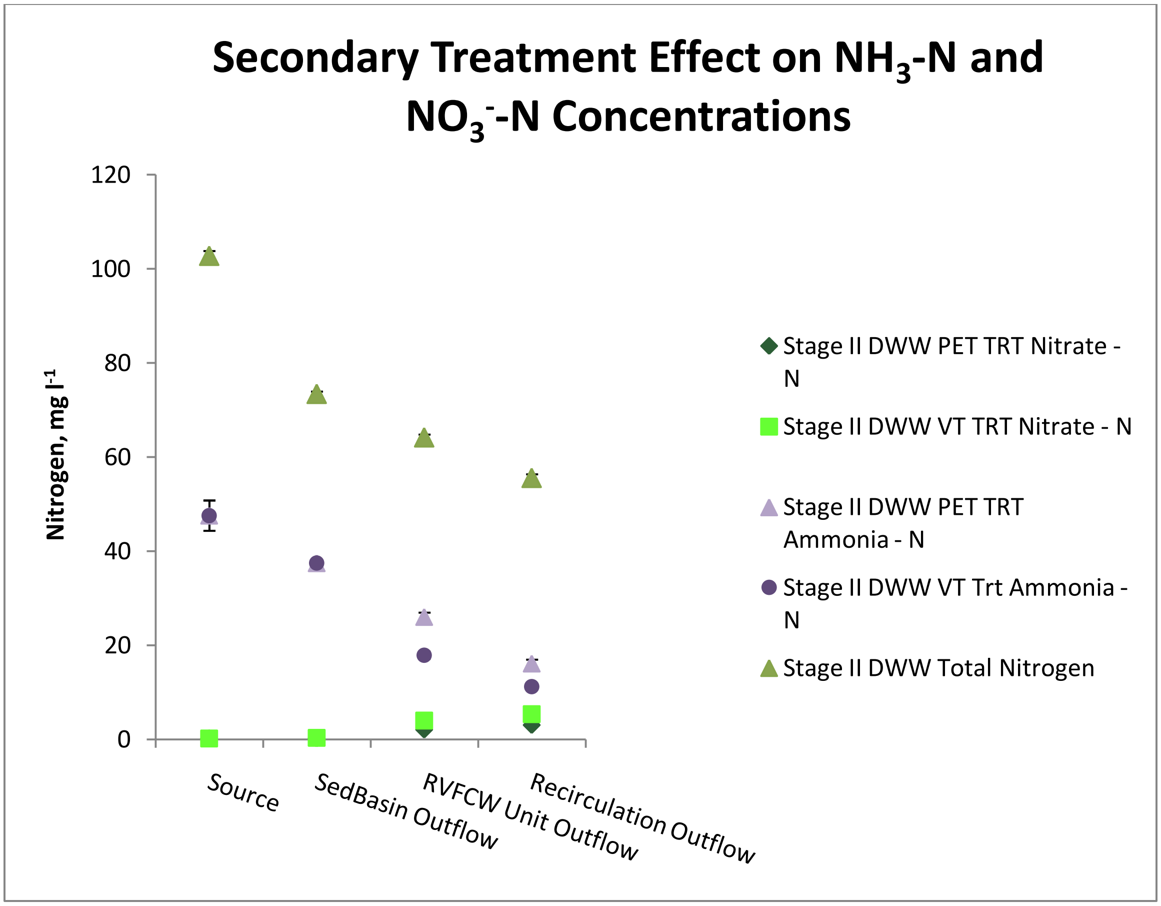

Figure 4.1: Comparison of recycled vertical flow constructed wetland type, and concentrations of total nitrogen, ammonia-nitrogen, nitrate-nitrogen concentrations, with standard error at the four sampling points (source, sedimentation outflow, RVFCW unit outflow, recirculation outflow) during dairy wastewater treatment.

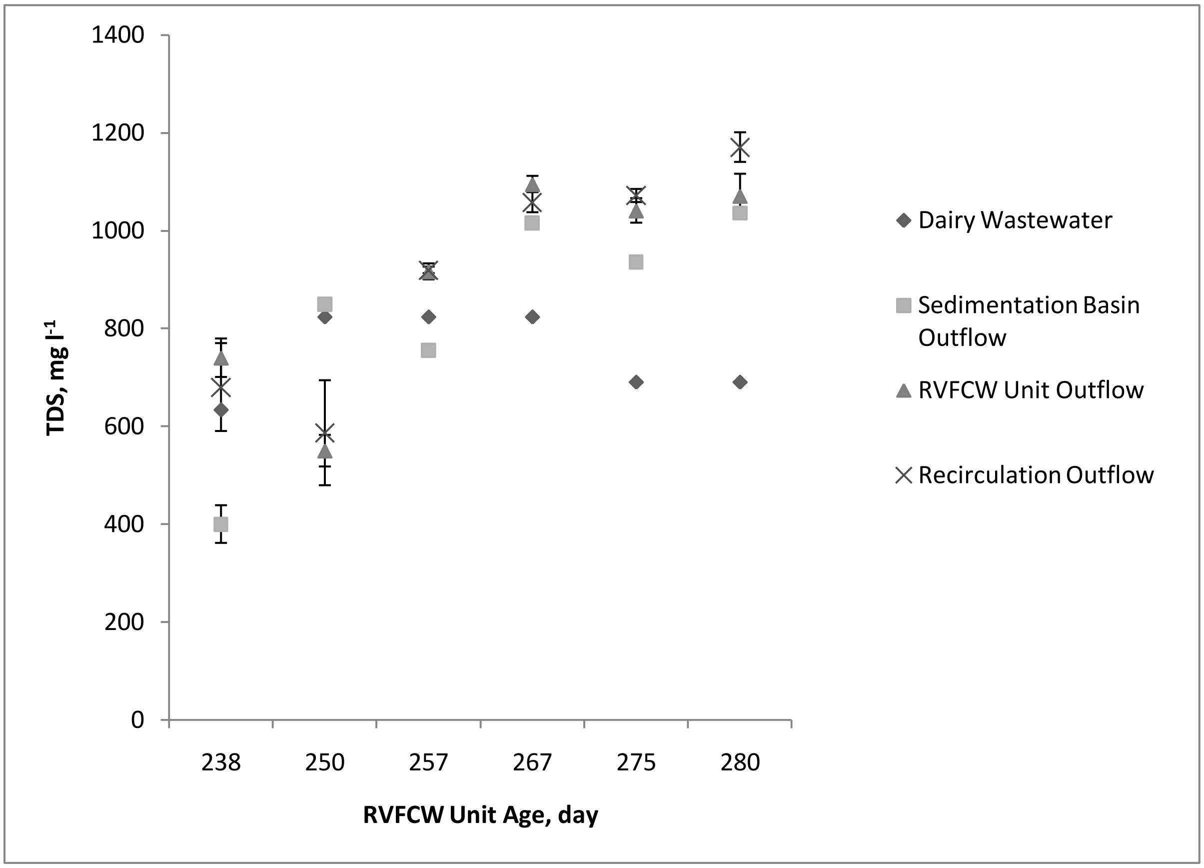

Figure 4.2: Total dissolved solids concentration, with standard error, over time at each sampling point (source, sedimentation outflow, RVFCW unit outflow, and recirculation outflow), during dairy wastewater treatment

Chapter 5

Summary

The overall performance of the RVFCW units indicate their potential as primary and secondary treatment systems for both greywater and dairy wastewater for potential reuse as irrigation water, livestock drinking water, and bathing water. Although the current design of the RVFCW units prohibit their use as a stand-alone domestic greywater treatment system, as was evidenced by the highly variable effluent concentrations of total plate count, fecal coliforms which exceeded some irrigation standards, hydraulic design modifications could ensure a more consistent and higher quality effluent (WHO, 2003).

Although the RVFCW units demonstrated exceptional treatment efficiencies during treatment of high strength dairy wastewater, the effluent levels of many target parameters remained high, indicating that the RVFCW units should be used in conjunction with another treatment system, such as horizontal subsurface flow constructed wetlands. Potential exists to improve removal efficiencies during dairy wastewater treatment if the recirculation rates and times are manipulated to produce additional contaminant removal above that which was observed during this research. In addition, adding another media layer between the topsoil layer and the middle VT or PET layer could aid in reducing the variability of the RVFCW's performance.

The high oxygen transfer rate, recirculation capabilities, and above ground design are substantial benefits of this RVFCW design. These attributes facilitate portability, making the designs better suited for used as emergency treatment systems in the event of municipal infrastructural damage due to natural disasters. The decreased land conversion need of the RVFCW units makes them a better economically and environmentally sustainable wastewater treatment system for impoverished communities, where are often characterized by high human and animal population densities. Also, the above ground design would facilitate the implementation of constructed wetlands in areas where shallow groundwater levels and seasonal flooding would have discouraged the use of constructed wetlands, or greatly impeded CW treatment efficiencies (Kadlec and Scott, 2009, Bajpai and Tripathi, 2000).

The use of recyclable materials in wastewater treatment systems is still vastly underdeveloped. Polyethylene terephthalate plastic is ubiquitous, found worldwide in the form of billions of discarded beverage bottles. Bottles are filling up landfills in parts of the world, and are nuisance pollutants in others. Polyethylene terephthalate plastic bottles represent a significant wastewater treatment resource as artificial wetland bed media. The results of this research indicate that the RVFCW PET achieved similar removal efficiencies to the RVFCW VT units. The RVFCW PET removal efficiencies for ammonia-nitrogen, and outflow concentrations of nitrate-nitrogen indicate that the PET media facilitated the development anoxic conditions. These results suggest that the RVFCW PET units would be better treatment systems for wastewater characterized by high nitrogen concentrations, such as agricultural effluent. In addition, the significantly lower capital cost associated with using recycled PET plastic as a the RVFCW bed material, suggest that when combined with a simple filtration system, such as the one described by Colwell et al. (2003), the RVFCW PET units could provide safer drinking and bathing water for those currently without access to adequate sanitation.

Further research is needed to determine the durability and life span of PET plastic in a wetland, and the risk to human reproductive health from endocrine disrupting compounds (EDC) leaching from the PET plastic segments. Research has been provided that indicates water stored in PET plastic bottles has an estrogenic activity three times higher than water packaged in glass (Wagner and Oehlmann, 2010). The estrogenic activity is due to the leaching of plasticizers (bisphenol A , phthalates, alkylphenols) from the PET plastic into the water (Wagner and Oehlmann, 2009). Risk of long term reproductive health effects from EDCs in the RVFCW PET unit effluent needs to be assessed. However, the acute risk of disease and death from water borne pathogens may outweigh long term risks of EDCs, if a constructed wetland built with PET plastic is the only affordable wastewater treatment option available to an impoverished community.

Design modifications of the current RVFCW design is needed, with subsequent performance evaluation is needed to determine the ability of the RVFCW PET units to act as a stand-alone wastewater treatment system for water recycling and reuse. However, the UN's goal of reducing the world's population without access to adequate sanitation should be a priority, and the RVFCW PET units developed for this research should be considered a valuable tool for reaching this goal (United Nations, 2008).

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